1974

The distribution and accumulation of mercury, lead, and cadmium in selected species of the northern California intertidal mussel bed

Vijay Kumar Khanna
University of the Pacific

THE DISTRIBUTION AND ACCUMULATION OF MERCURY, LEAD, AND CADMIUM IN SELECTED SPECIES OF THE NORTHERN CALIFORNIA INTERTIDAL MUSSEL BED

A Thesis

Presented to

the Graduate Faculty

of the

University of the Pacific

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Vijay Kumar Khanna

February 1974

This thesis, written and submitted by

Vijay Kumar Khanna

is approved for recommendation to the Committee on Graduate Studies, University of the Pacific.

Department Chairman or Dean:

Carl C. Purnell

Thesis Committee:

Bartlett D. Whitton Chairman

Dated February 21, 1974

ACKNOWLEDGMENT

I would like to express my sincere gratitude to Dr. Bartlett D. Whelton under whose supervision this work was carried out, for his encouragement and continued guidance. The various field trips and discussions we had during this work were pleasant and enriching experiences to me.

Thanks are also due to Dr. Marvin H. Malone for his interest, advice, and support.

Dr. William Gladfelter was very helpful in initial stages of the work.

I would also like to express my thanks to Mr. John McGowan, Mr. Lohit V. Tutupalli, Mr. Donald Peterson, and Ms. Sarah Saunders for their help and unfailing friendship.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

TABLE PAGE

TABLE

PAGE

LIST OF FIGURES

INTRODUCTION

Historically, the extraction and utilization of lead dates back to the beginning of recorded time while that of mercury can be traced to at least as early as 1100 B.C. Cadmium, on the other hand, has not been utilized intentionally by man until this present century. In recent years these heavy metals have been labelled as being hazardous to human health. The main cause for alarm concerning heavy metal contamination has been due in a large part to a more critical evaluation of an increasing number of man's own technological advancements, the development of which have been exponental since the industrial revolution. An increasing number of industries are using these metals as raw materials, catalysts, etc. The large consumption of these metals results in a discharge of potentially dangerous waste materials into our environment; thus soil, water, and air have accumulated relatively high levels of these metals.

Although sea water is not usually directly consumed by man, it is responsible indirectly for a certain portion of the increasingly higher levels of these metals found in human tissues, since those marine animals which we consume have appreciable amounts of these metals apparently acquired from sea water itself.

The mussel bed and its multitude of inhabitants form a life community typical of our intertidal, rocky, open-coast areas. These animals are essentially immobile, are conveniently available at low

tide, and have been well defined in their consumer order. Certain main members from this community were chosen with the intention that they would represent an index of heavy metal pollution for a given area under different seasonal and other variable conditions. Samples for monitoring were collected from two different sites. The first site was immediately outside the entrance to San Francisco Bay and located between Seal Rocks and Phelan Beach State Parks. This location was chosen to represent a water mass of supposed maximum pollution. The Golden Gate can be assumed to be the funnel through which flows all waters from the San Joaquin and Sacramento River drainages and from the San Francisco Bay area itself. The second site, immediately north of the Dillon Beach township, located at the juncture of Bodega and Tomales Bays, was chosen since it might represent a water mass of minimum pollution. This area is not immediately near any large urban influence, industrial activity or subject to heavy auto traffic. Therefore, at the outset it was hoped that the "immobile" consumer order within the mussel bed community would reflect the relative pollution of two supposedly different water masses.

In the first phase of the project, a ten-month monitoring of lead, cadmium, and mercury in the mussel bed was accomplished in order to establich a base line with respect to any variation in season, rainfall, feeding habits, offshore currents etc. Also of interest was a correlation of respective metal levels in tissue with regard to their pyramiding through the consumer order based on wet and dry tissue weight and on tissue protein content. In the second phase an

attempt was made to determine the origin of the heavy metals in certain of the selected animals from the consumer chain with respect to accumulation (absorption and/or adsorption) from sea water or feeding or both.

Figure 1 depicts that portion of the mussel bed consumer chain of interest in this work:

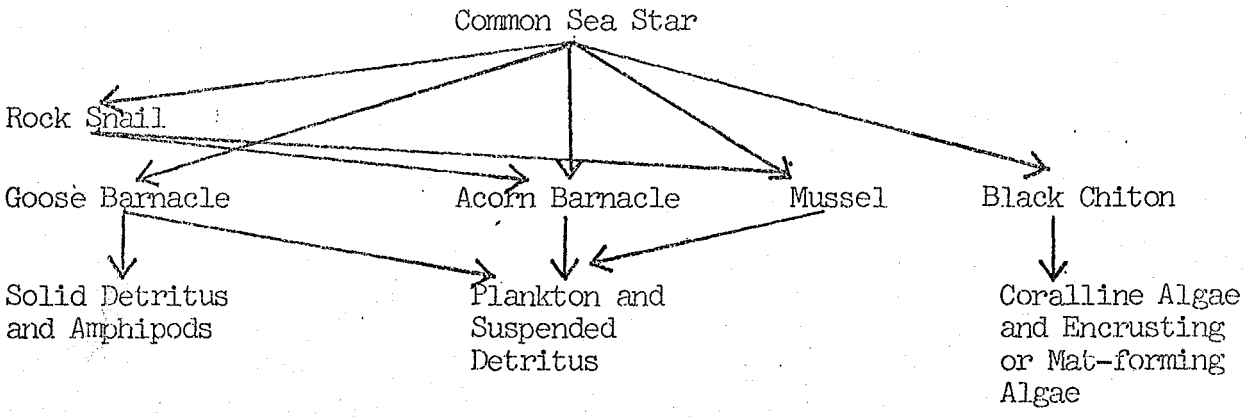

Figure 1

A good basic survey of the life processes within the mussel bed is presented in the book, Between Pacific Tides, by E.F. Ricketts and J. Calvin (1968), and as such has been extensively consulted in the following general description of the animals of interest in this work.

The most conspicuous of the rocky, open coast animals is the ochre sea star, Pisaster ochraceus, commonly but improperly called

the starfish. Its range in the intertidal zone covers the high (+5 to 2.5 feet above sea level), middle (+2.5 to 0 feet) and low (0 to -2 feet) zones - all but the uppermost or splash (+7 to +5 feet) zone. Specimens vary from six to fourteen inches in diameter and have three color phases - brown, purple, and yellow or orange. They are commonly seen on or near the mussel beds of exposed rocks at low tide. Pisaster ochraceus, a tertiary consumer, devours any other animal it can capture within the mussel bed community. It can evert and thrust its cardiac stomach either into the shell of a snail or acorn barnacle, or around a chiton, limpit or goose barnacle. A sea star is occasionally observed humped up over a mussel apparently pulling its shell apart in order to insert its stomach into the victim. In short, the prey of the sea star is governed somewhat by the former's availability and mobility. Thus, where there are barnacles, it will eat barnacles, although they are of low nutritional value. Where there are mussels or limpits, animals of higher food value, it will also consume them (Paine, 1966; Feder, 1959; and Menge, 1972).

In general, the sea star is known to be a more active feeder during high tide rather than at low tide, and he has an annual cycle of feeding - characteristically low in winter (the breeding period for females) and high in the summer (Menge, 1972). The sea star sits at the apex of the consumer chain in the mussel bed (see Figure 1) and as such is subjected to no other predator in nature other than man.

The sea star's respiratory and excretory systems have yet to be well defined. However, it is believed that the tube feet as well

as the finger-like extensions (called the dermal branchiae and lined inside and out with cilia) covering the animals upper surface all play a role (Storer et al., 1972).

The small, carnivorous snail, Thais emarginata, is found throughout the high, middle, and lower intertidal zones. This secondary consumer feeds by drilling holes with its radula through the shells of small mussels and acorn barnacles and extracting their soft tissue bit by bit - a process requiring about 10.5 hours. Thais is known to decrease or stop feeding from November through February and then resume this activity from March through October. Peak feeding is reached during August and early September (Connell, 1970). This animal is cannibalistic; the adults eat snail eggs indiscriminately while the larvae themselves consume each other within the egg sac until one is left to emerge.

On account of its relatively thin shell, this species of Thais, when venturing to the lower tidal zones, probably also falls prey to large crabs and fish as well as to the sea star for which it has not developed or evolved an "escape response" (Connell, 1970).

The California mussel, Mytilus californianus, a rocky, open coast bivalve ranging from Alaska to Mexico, forms great intertidal beds that are located predominately in the middle intertidal zone and, to lesser extents, in the high and low zones.

As a primary consumer, the mussel filters minute organisms or suspended material from seawater. The mussel may achieve limited locomotion in the bed by alternately anchoring itself to the rock

surface with its tough byssal hairs (extruded by a gland in the foot) and then extending its foot to grip a new portion of rock thereby breaking or pulling out the attached byssal hairs (Harger, 1971, 1972).

The black chiton, Kathrina tunicata, is a primary consumer usually found only in the low intertidal zone of heavily surf-swept rocks of the open coast. As a herbivore, the animal grazes on encrusted algae from the rock surface, and its stomach contents also have been found to contain coralline algae, Corallina chilensis, a type of algae or kelp common in the lower middle intertidal zone (W. Gladfelter, personal communication). The black chiton's high caloric value makes it a desirable catch for the sea-star. However, since these animals are mobile and are not found in large numbers, they do not often fall prey to the sea star (Feder, 1959; Menge, 1972).

The goose barnacle, Pollicipes polymerus, lives in clusters attached to rock surfaces, situated in the high and especially in the middle intertidal zones. This barnacle feeds on amphipods and solid detritus carried to it by surf action. Each member of the cluster is aligned with the direction of the wave or current movement, and each is capable of slowly bending or twisting its stalk to alter the group orientation - a maneuver which may have significance in capturing food.

Although in physical appearance the acorn barnacle, both Balanus glandula and cariosus, is strikingly dissimilar from the goose barnacle, the two are closely related and follow much the same life history. This

animal inhabits the rock faces of the high intertidal region. Identified by its miniature volcano shaped shell, the acorn barnacle, when covered by a wave, will open its operculum and sift the sea water with its thoracic appendages as a means of both respiring and securing phytoplankton.

Finally one other of the hordes of inhabitants of the mussel bed proper must be considered -- the porcerlain crab, Petrolisthes cinctipes. This animal takes advantage of the shelter provided from crashing waves by the mature mussel community by foraging and scavenging among their byssal hairs for plankton, suspended detritus, etc. The carapace of this flat reddish crab is usually not much longer than a person's fingernail, and this small size allows it considerable freedom of movement in tight quarters. The animal is also found beneath rocks in the high and middle intertidal zones.

The following authors have been extensively consulted in presenting the following discussion of the absorption, distribution, toxicity, and excretion of mercury, lead, and cadmium: Vallee and Ulmer, 1972; Clarkson, 1972; Lee, 1972; and Goodman and Gilman, 1971.

The scientific advances of this age are largely responsible for the environmental pollution we are facing today. For example, the use of the mercurial fungicides has increased mercury levels in agricultural soil and the water draining that soil. A major source of lead in the environment is that of automobile exhaust, and to a lesser degree that derived from paint, solder, shotgun pellets and storage batteries. Cadmium finds its way into the pollution picture

as a result of its use in automotive electroplating, in pigments and plastic stabilizers, and in nickel-cadmium batteries, semiconductors and photocells. Thus with either their dissemination or disposal in the environment these three metals - as well as other pollutants - ultimately find their way to our fresh and salt-water sources. On entering a given water mass they may be assimilated by the various animals and plant life indigenous to that area. For example, it has been reported that marine algae can accumalate one hundred times more mercury than is present in seawater (Vallee and Ulmer, 1972). In turn, algae, the primary producers, are then consumed by other marine animals, ultimately ending in the food chain with man.

Some twenty years ago, the fish-eating residents of Minimata Bay, Japan were subjected to severe and even fatal mercury poisoning. The source of the tragedy was an industrial discharge from a plastics manufacturing plant which, in turn, contaminated the fish life of the bay with both inorganic and methyl mercury residues. At about this time in Sweden a rapid decline in fish-eating birds was observed. Moreover, the birds consuming seeds treated with mercury fungicides were also dying. Different forms of mercury were linked to these fatal contaminations. Thus, as these examples indicate, the main danger to our environmental integrity is the discharge of wastes and/or other agents containing these toxic agents into air, water, and soil.

The distribution, absorption, and toxicology of mercury in biological systems is dictated primarily by the form in which it is acquired. Mercury may be present in industrial discharge as inorganic

or organic mercury. The different forms that constitute inorganic mercury are metallic mercury (Hg°), and divalent, ionic mercury (Hg^{++}). Organic mercury may be persent as methyl mercury (CH_3Hg^{+}), Phenyl mercury ($C_6H_5Hg^{+}$), or alkoxyalkyl mercury ($CH_3\text{-}O\text{-}CH_2\text{-}CH_2\text{-}Hg^{+}$).

Of these mercurials, methyl mercury is the most toxic to biological systems. Because of its high lipid solubility, methyl mercury can cross the blood-brain barrier and cause central nervous system (CNS) damage. Even the placenta does not offer any barrier to methyl mercury. Studies with experimental animals have indicated that the fetus contained higher levels of methyl mercury than the brain tissue of the mother. Thus methyl mercury may result in CNS damage to either or both the mother and unborn baby. Other forms of mercury are as potentially toxic as methyl mercury, since under certain conditions they can be converted to methyl mercury by enzymatic and non-enzymatic reactions.

Methionine synthetase catalyzes the conversion of homocysteine to methionine; methylcobalamin acts as a coenzyme in this reaction. The enzyme methionine synthetase may be present in many aerobic and anaerobic bacteria and in the liver of mammals. It can also be assumed that the vitamin B_{12} analog, methyl cobalamin, may be present in several organisms found in the bottom sediments of rivers, lakes, and oceans and in mammalian liver tissue. Several authors have suggested and shown in isolated systems with low concentrations of mercuric ion and under anaerobic conditions that methyl cobalamin is capable of transforming methyl groups as the methide carbanion ($:CH_3^{\circ}$) to Hg^{+2} to produce the methyl and dimethyl mercury derivatives. It was also shown

for these enzymatic and nonenzymatic pathways that dimethyl mercury is synthesized first and then probably decomposes to methyl mercury. The metal in this form can be taken up in all living ecosystems and eventually exert its toxic effects at all consumer levels.

When taken orally, soluble salts of inorganic mercury are readily absorbed into the blood stream as the divalent mercury ion. In the case of the very insoluble mercurial salts, e.g. calomel (Hg_2Cl_2), a certain portion can be oxidized to the more soluble mercuric form which can then be absorbed from the G. I. tract. However, the major portion of the insoluble mercurials are excreted unchanged in feces. Once in the blood, most of the divalent mercury is accumulated in the kidneys, and to a lesser degree in the liver, bone-marrow, spleen, intestinal wall, skin, etc. Even though its level in most organs may fall due to excretion, the level of divalent mercury generally continues to rise in the kidneys. In the kidneys, an excess of divalent mercury seems to promote the synthesis of metallothionein, by combining with a sulfur-rich protein, thionein. Metallothione may play some protective role, since it has been noted that mercury's toxicity to renal tissue becomes pronounced only when all thionein has been saturated. This protein is also present in human liver and that of other animals (liver and kidneys of rabits, kidneys or rats and horses). However, in contrast to cadmium and zinc, mercury seems to form metallothionein only in the kidneys.

Elemental mercury can be inhaled or absorbed through the skin. When inhaled, elemental mercury is well absorbed through the cell

membranes of the lungs. Due to its lipid solubility, elemental mercury can also cross the blood-brain barrier and accumulate in the cerebral tissue. Here it may be oxidized to divalent mercury before binding to protein and/or exerting its toxic effect. Small amounts (0.02-0.04% w/v) of ethanol have been reported to inhibit approximately 30% of the absorption of elemental mercury vapor in the lungs in humans. The mechanism for this action of ethanol is yet unknown. (Clarkson, 1972).

The rate of the excretion of various mercurial derivatives depends upon the actual form in which this metal exists in the tissues of different animals. Generally the excretion of divalent mercury starts immediately after its absorption, mainly through urine and feces. Most of the metal may be excreted in the first few days though traces may continue to be found in urine for many months. Organic mercury compounds like phenyl mercury, methoxyethyl mercury, etc. are excreted at a nearly identical rate to that of divalent mercury. However, methyl mercury salts are more slowly excreted than any of the other derivatives. In humans, it's half-life is about seventy days -- thus one aspect causing methyl mercury to be more toxic to biological systems than other mercurials.

Mercurials, as well as the compounds of lead and cadmium, ultimately exert their toxic effect by binding with mercapto, amino, imino, carbosylate, etc. groups of proteins and enzymes. This may result in changes of the specific functions of these proteins in living systems, (e.g. in the cell wall) and may reduce if not inhibit the activity of enzymes.

Certain organolead derivatives (tetraethyl and tetramethyl lead) are added to gasoline to act as anti-knock agents. Most of these are converted to lead halides in the automobile engine, and they are emitted in particulate form. These halide particles constitute the main airborne lead hazard to which we are directly exposed. Only a small fraction of organic lead (10%) is emitted from the auto engine unchanged. Yet this form of the metal is more toxic than other types because it can be absorbed rapidly through the skin or lungs, cross the blood-brain barrier and thus exert its toxic effects on cerebral tissue.

Almost half of the particulate lead halide incorporated in man is inhaled; the other half may be swallowed. Approximately half of the inhaled lead remains in the lungs while the remaining portion by some undefined route reaches the alimentary tract. A small amount (approximately 10-12%) of this lead is absorbed into the gut (Kehoe, 1964). Swallowed particulate lead lends to follow a distribution pattern similar to ionic lead described in the following paragraphs. It can be noted here that when metallic lead dust is inhaled, it must be oxidized to the ionic form before it can be absorbed and bound to red blood cells.

Foods and beverages are the main source of other inorganic lead salts in the human body. The average intake of lead for healthy individuals in the United States from these sources is about 0.30 mg/day. As with the halides, only a small portion of this lead (5-15%) is absorbed from the G. I. tract and passed into the blood circulation,

the rest being excreted through the feces. From the blood, the metal is distributed initially to the soft tissue with maximum concentrations found in the kidneys and lesser amounts in the liver. In time, part of the metal is redistributed to the bones, teeth and hair.

Lead and calcium are ion exchangable, and with most organic and inorganic ligands, lead has a far greater binding capacity than does calcium. This leads to increased levels of lead in bones and teeth with age. The metal may be present at levels of 90 PPM in teeth of people over fifty years of age. But this high level should not be considered an index of body burden of lead as teeth may exchange lead directly from the external environment (contact with food). Lead present in bones and teeth is in a generally non-diffusable and immobile form, therefore, and is relatively non-toxic. It is that metal present in the blood, brain, and kidneys that interfers with regular body functions and causes toxic effects.

Ionic lead, like other heavy metal ions, has a high affinity for the mercapto, carboxyl, amino, etc. groups of proteins and enzymes. Such binding or complexation may result in partial or complete enzyme inhibition by blocking the active site and/or by promoting a conformational change that is unfavorable for catalysis.

Ionic lead appears to interfere with heme synthesis in bone-marrow, thereby reducing the oxygen carrying capacity of blood. It may also lead to an increased rate of destruction of red blood cells. A combination of these two effects probably is responsible for the anemia which is observed in humans with lead poisoning.

Unlike methyl mercury, part of the organic lead is converted to inorganic lead in the body. As previously stated, organic lead may cross the blood-brain barrier causing severe brain damage. On decomposition to the ionic form, it is possible that its presence as such may also interfere with impulse transmission probably by replacing calcium ions at the motor end plate of the nerves.

Ionic lead present in the blood is excreted mostly through the kidneys. It may result in a reduced capacity of the kidneys to re-absorb amino acids, glucose, uric acid, citric acid and other small organic molecules.

Due to recent and strict antipollution regulations restricting the quantity and form of lead in gasoline, the danger of environmental contamination by lead has been considerably reduced. However, children may still be victims of lead poisoning by chewing lead-based paints stripped from the walls of old homes. Occasional cases of poisoning arise from the use of pottery finished with a lead glaze for the cooking and storage of food.

The use of sensitive analytical tools such as atomic absorption spectroscopy has indicated that cadmium is present in trace quantities in practically all the food and beverages consumed by man. Industrial discharge is not completely responsible for this since it was only a few years ago that this metal found its importance in industry. It is probably the natural distribution of this element in soil and water that is mainly responsible for its presence in edible materials. With the exception of ore miners, very few clinical cases of cadmium

poisoning have been encountered. Thus most of our knowledge of its toxicology has been obtained from studies done on experimental animals.

The effects of organic cadmium on experimental animals has not yet been investigated. This is in all likelihood due to the fact that the alkyl derivatives of cadmium are very reactive and unstable in aqueous media. However, if certain organocadmium derivatives are sufficiently stable to exist in the environment, then their alkylating capacity could lead to a significantly different toxicity than that of ionic cadmium.

Inhaled elemental cadmium fumes are transported from the respiratory tract to other soft tissues within a few days, however, the metal must first be oxidized to the ionic form before protein and/or other tissue binding can occur.

When orally or parenterally administered to animals, the soluble inorganic salts of cadmium have been shown to accumulate in the hepatic and renal tissues. It is also known that cadmium competes with zinc for the mercapto groups of the protein thionein found in these tissues. More cadmium than zinc is found to be bound in metallothionein (in horses 5.9% cadmium, 2.2% zinc; in humans 4.2% cadmium, 2.6% zinc) indicating that cadmium binds more firmly than zinc in this protein.

This property of cadmium to accumulate in the kidneys has drawn the attention of several investigators attempting to relate hypertension to cadmium toxicity. Numerous research papers have been published but no conclusive evidence was found to prove the theory.

In humans, and in experimental animals subjected to chronic cadmium exposure, renal tissue damage and urinary excretion of small molecular weight (approximately 10,000-200,000)proteins have been observed. The bulk of these proteins are thought to be the α^2, β and γ globulins (albumin to a lesser degree).

In Japan, a condition called Itai-Itai (ouch-ouch) disease was observed in elderly women. It was characterized by a high urinary excretion of calcium ions, glucose, low molecular weight proteins, amino acids, etc. A cadmium mine in the vicinity was suggested as a cause for the malady; however, due to the lack of sufficient evidence and its non-occurance since 1955, the correlation of the Itai-Itai syndrome with cadmium toxicity has not been established conclusively.

There is little published data on the mechanisms of heavy metal toxicity in marine animals, although a few suggestions have been forwarded. It is believed that heavy metals accumulate mainly in the gill tissue of such animals, thereby imparing their ability to respire freely. A reduced oxygen supply to the internal organs may result in reduced hepatic and cardiac function, altered metabolic rates and may lead finally to death. (Pringle, et al., 1968); (Eisler, 1971).

EXPERIMENTAL AND MATERIALS

One of the main reasons for the previous lack of data on trace metal analysis in biological systems was the absence of specific, sensitive, accurate, and rapid techniques for their determination. Many of the earlier techniques involved a long and tedious precipitation procedure followed by chelate extraction and finally ultraviolet or visible spectrophotometric analysis. The classical method for the determination of trace metals in biological systems has been the dithiazone chelate extraction method. Although its specificity is not good, it can be improved by the proper choice of organic solvent and the pH of the aqueous phase for the extraction portion of the experiment. Also, the sensitivity has not proven satisfactory in all cases (Sandell, 1959). Neutron activation analysis can be used to estimate most of the elements of the periodic table (Sorte, et al., 1972). Several workers have used this technique to determine mercury in the organs of birds and animals and in human blood, urine, and tissues (Johnel and Westermark, 1969) (Sjostrand, 1964). Though this method enjoys the advantage of being specific and sensitive, its main disadvantage is in its expense and safety requirements.

On the basis of accuracy, specificity, speed, and sensitivity atomic absorption spectroscopy presently is the method of choice for trace metal analysis. In this technique the metal of interest is reduced to its ground state atomic form (usually by means of a flame) so that the atom (lacking both vibrational and rotational energy modes in the molecular sense) is capable of absorbing radiation emitted by a hollow cathode lamp and undergoes only electronic excitation (Kahn, 1967; Robinson, 1970). This lamp is filled with argon or neon

gas at low pressure which becomes ionized at its anode and then is accelerated toward a cathode composed of the element under consideration. On bombardment the surface metal atoms are "boiled off" in their excited state and give rise to an emitted electronic spectrum characteristic of that element and composed of very narrow spectral lines, (approximately 0.02 Å wide). As such the lamp when coupled to the appropriate monochrometer and a system of slits becomes a source of essentially monochromatic light corresponding to the desired element, thus making atomic absorption a very specific technique for trace analytical work.

Metallic mercury, however, does not lend itself well to the conventional flame atomic absorption technique as it lacks the sensitivity required for trace analytical work (15 ppm for 1% absorption). (Analytical Methods for Atomic Absorption Spectrophotometry, Perkin Elmer).

The more recently developed flameless technique used for mercury analysis takes advantage of the fact that organic and inorganic mercury compounds can be converted easily to atomic mercury (Hatch and Ott, 1968). In general, mercurial derivatives are oxidized yielding mercuric ions which are then quantitatively reduced by the addition of stannous chloride as indicated in the following equation (Kimura and Miller, 1962):

$$Hg^{2+} + Sn^{2+} \longrightarrow Hg^{\circ} + Sn^{4+}$$

The reduced atomic mercury is aerated from its solution in a closed mercury analysis system (supplied by Perkin-Elmer). In this system the aerated atomic mercury passes through a cell positioned

in the light path of the hollow cathode lamp in the atomic absorption spectrometer. The absorbance thus measured is compared to standard curves and directly corresponds to the amount of metal in the reaction vessel. With such a technique mercury is detectable down to levels of 1 ppb.

For other metals, such as lead and cadmium which have reasonable sensitivity with the flame technique, the non-specificity of organic chelate extraction is more than overcome by the specificity achieved in utilizing the essentially monochromatic radiation emitted by the hollow cathode lamp in the atomic absorption spectrometer. Thus in chelate extraction the chelating agent need only be efficient (large binding constant) but not necessarily selective, since other trace elements generally do not interfere with the analysis for the given element in question. Heretofore, the use of ultraviolet or visible spectrophotometric techniques required chelate efficiency as well as specificity - conditions seldom met - since the absorption band width of chelate complexes (due to combinations of electronic excitement with vibrational and rotational modes) were comparatively large (on the order of tens of angstroms) and, therefore, overlapping absorption bands "Interference" could hardly be avoided. For this study, sodium diethyldithiocarbamate (NDDC) and ammonium pyrrolidinedithiocarbamate (APDC) were used as chelating agents for the metals cadmium and lead respectively (Berman, 1967; Sprague and Slavin, 1964). The sensitivity for cadmium by the flame technique is about 0.03 PPM for 1% absorption and for lead is about 0.5 PPM for 1% absorption. (Analytical Methods for Atomic Absorption Spectrophotometry, Perkin-Elmer).

Apparatus:

Perkin-Elmer 290 Atomic Absorption Spectrophotometer
Sargent Recorder Model # DC-1200
Perkin-Elmer Mercury, Lead, and Cadmium hollow cathode lamps
Perkin-Elmer Mercury Analysis System (303-3119)
Burrell Wrist Shaker Machine

Instrument settings for the Perkin-Elmer 290:

Lamp current --- 4 m.a.
Wave length setting - Hg, 2357Å; Cd, 2088Å; Pb, 2833Å.
Slit setting -- 7Å

Recorder settings:

Chart speed - 1 inch per minute
Range - 50

Chemicals: (All reagents and chemicals used were of Reagent grade).

Sulfuric acid, 36N and 18N solutions
Nitric acid, 16N, 5.6N and 5% solutions
Hydrochloric acid, 10% aqueous solution
Perchloric acid, 70%
Sodium hydroxide, 2.5% aqueous solution
Ammonium hydroxide, 2.5% aqueous solution
Potassium permanganate, crystals and as a 5% aqueous solution
Hydroxylamine hydrochloride, crystals and as a 1.5% aqueous solution
Stannous chloride, 10% aqueous solution
Magnesium perchlorate, (drying agent)
Silica gel; Activated carbon; Glass wool
Sodium diethyldithiocarbamate, 1% aqueous solution (NDDC)
Ammonium pyrrolidinodithiocarbamate, 1% aqueous solution (APDC)
Methylisobutylketone (MIBK)

Standard Solutions for Atomic Absorption:

Mercury standard solutions were made from anhydrous $Hg(NO_3)_2$

Cadmium standard solutions were made from anhydrous $CdSO_4$

Lead standard solutions were obtained from the Fisher Scientific Company as 1000 PPM Pb as $Pb(NO_3)_2$ in dilute HNO_3.

Miscellaneous:

Double distilled water was used for preparing all solutions.

Instant Ocean mix was supplied by Aquarium Systems, Inc., Ohio.

Standard Solutions and Curves

A stock mercury solution (1mg Hg/ml) was prepared by dissolving 1.618 g of anhydrous mercuric nitrate in a 1000 ml volumetric flask and diluting to volume with a 5% nitric acid solution.

Four ml of stock solution were diluted to the 1000 ml mark in a second volumetric flask with 5% nitric acid. By diluting 2.5 ml of this solution to 100 ml in a third volumetric flask, a working mercury standard was prepared. This solution contained 0.1 mg of mercury per ml of solution. Various volumes of the working mercury solution were prepared in biochemical oxygen demand (B.O.D.) bottles (Table I) for the standard curve as follows (Thorpe, 1971). A blank solution was also prepared to which no mercury working standard was added. To each of the B.O.D. bottles two drops of 5% potassium permanganate solution were added followed by 5 ml of 5.6N nitric acid and 5 ml of 18N sulfuric acid respectively, with swirling of each flask after each addition. After waiting for a minute, 5 ml of 1.5% hydroxylaminehydrochloride were added. This solution usually rendered the contents of the bottle colorless. If it did not, another ml or two were added until the solution in the B.O.D. bottle turned colorless. Finally, 5 ml of 10% stannous chloride were added and immediately the aeration probe of the mercury analysis system was inserted into the B.O.D. bottle. The resulting absorption reading from the atomic absorption spectrometer was then recorded and plotted versus the known mcg of mercury and this served as the standard mercury curve. (Instructions for Mercury Analysis, Perkin-Elmer). For each set of the monitoring and accumulation data, the standard curve was repeated before and after each study was performed to insure accuracy. This was done for each of the metals.

Table I

Distilled Water in B.O.D. Bottle (in ml)	Aliquots of working std. (in ml)	Mercury in Reaction Vessel (in mg)
79.0	1.0	0.1
77.5	3.0	0.3
75.0	5.0	0.5
72.5	7.5	0.75
70.0	10.0	1.0

The cadmium stock solution was prepared by dissolving 1.8550 g of anhydrous cadmium sulfate in a volumetric flask and diluting it to the 1000 ml mark with 5% nitric acid solution. This solution contained 1 mg Cd/ml (1000 PPM). By diluting 1 ml of stock solution to 1000 ml in a volumetric flask with 5% nitric acid, a working standard containing 1 μg Cd/ml was prepared. Aliquots varying from 10 μg-0.50 μg total Cd were pipetted into 150 ml beakers. The pH of each dilution was then adjusted to around neutrality (6-7.5) by adding sufficient amount of 2.5 N sodium hydroxide. The metal ion was extracted as the chelate with 10 ml of methylisobutylketone (MIBK) after adding to the aqueous solution 1 ml of 1% sodium diethyldithiocarbamate (NDDC). The MIBK extract was collected in a 10 ml volumetric flask and made to volume with MIBK. Thus the final cadmium ion concentration in the various 10 ml volumetric flasks ranged from 1.0 μg/ml to 0.05 μg/ml in MIBK or from 10 μg to 0.5 μg in total Cd. The nebulizer tubing of the instrument was directly inserted into the volumetric flask containing the metal extract and the absorption level was recorded. A standard curve was drawn by plotting divisions of absorption from the instrument's meter against the known concentration of cadmium. On analysis of these standards, it was found that Cd at 0.05 μg/ml concentration was undetectable with our instrument.

For lead analysis, the commercially available atomic absorption lead standard containing 1 mg Pb/ml (1000 PPM) was used. One ml of this solution was diluted to 1000 ml to give a working solution of 1 μg Pb/ml. Aliquots of this working solution ranging from 10 μg

to 100 μg total Pb were pipetted into 250 ml beakers. Each aliquot was then adjusted to a pH of 2.8 with 5% nitric acid or 2.5% sodium hydroxide. Finally the metal ion was chelated by adding 1.0 ml of 1% ammonium pyrrolidinodithiocarbamate (APDC), extracted with 10 ml MIBK and collected in a 10 ml volumetric flask. The extract was made to volume with MIBK and directly subjected to atomic absorption spectroscopy in the same manner as for cadmium. Thus the final lead ion concentration in the various 10 ml volumetric flasks ranged from 1 μg/ml to 10 μg/ml in MIBK or from 10 to 100 μg in total Pb. The sample absorbance was plotted versus the known concentration of lead in the sample resulting in the standard curve for lead.

Examples of standard curves for mercury, cadmium, and lead are shown in Figures 2-4 on the following pages.

Collection and Storage of Specimens:

The specimens for the monitoring study were collected from a rocky area immediately north of the Dillon Beach township and from a rocky point east of Land's End roughly midway between Seal Rocks and Phelan Beach State Parks and located at the mouth of San Francisco Bay. The animals were collected well above (at least 3 feet) the water line. The sizes of the different animals are as stated in Table II. On collection, the animals were immediately frozen on dry ice and stored in a styrofoam cooler for transport to our laboratory at the University of the Pacific, Stockton. Then each specimen was rinsed, duly labeled, and then held in the deep freeze cabinet until

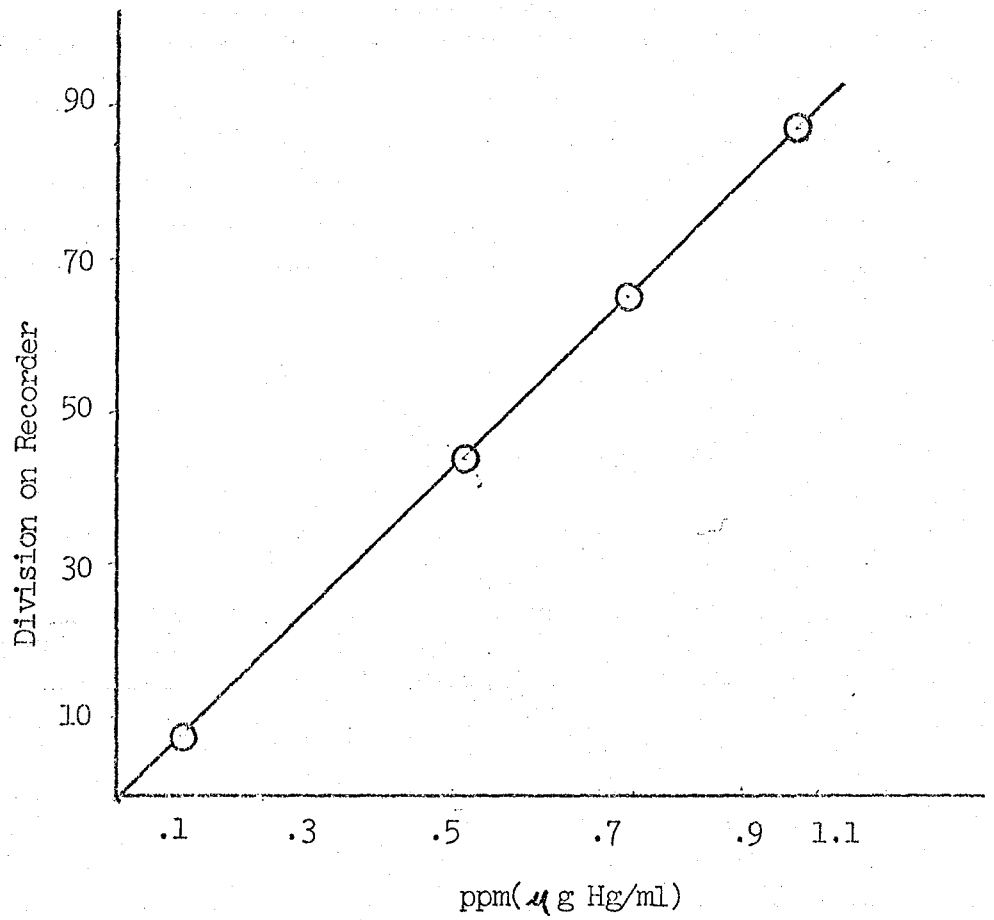

Figure 2:
Standard Curve for Mercury
Taken from Monitoring Series II from Dillon Beach
(Range 0.1 to 1.0 μg Hg)

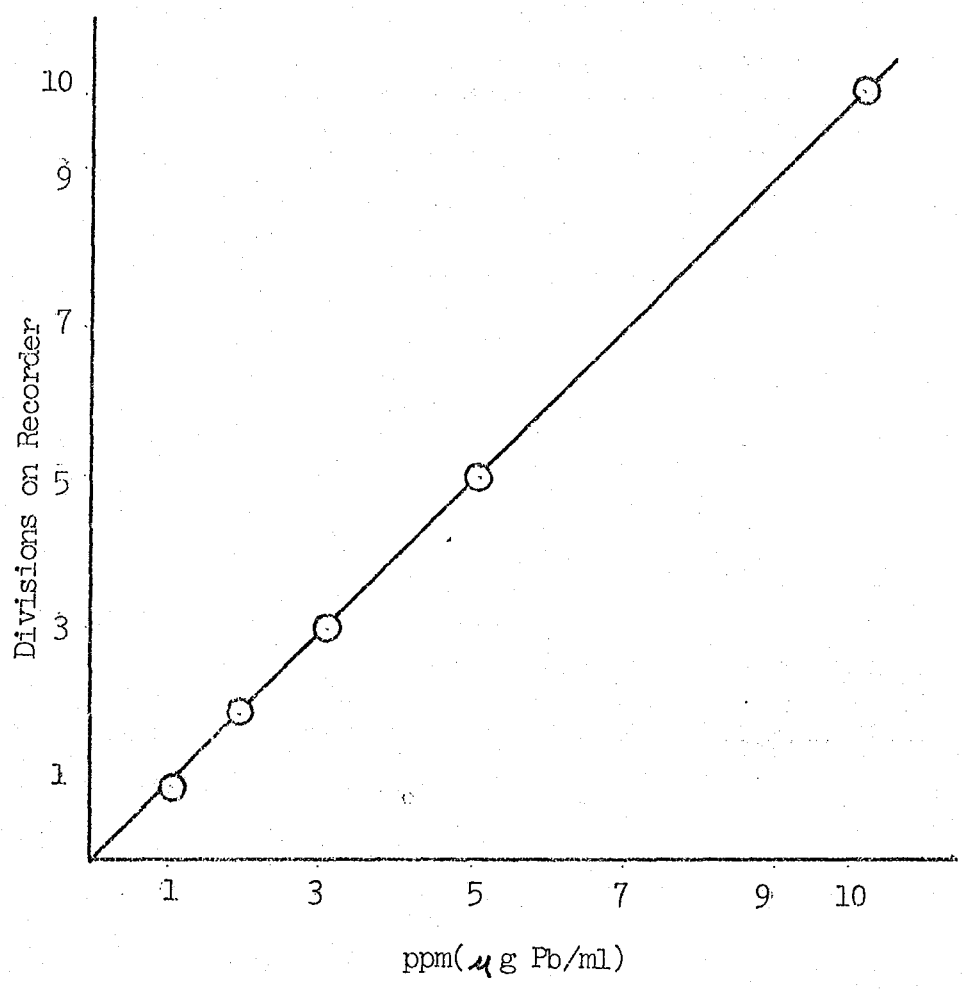

Figure 3:
Standard Curve for Lead
Taken from Monitoring Series VII from Dillon Beach
(Range 1.0-10.0μg Pb/ml)

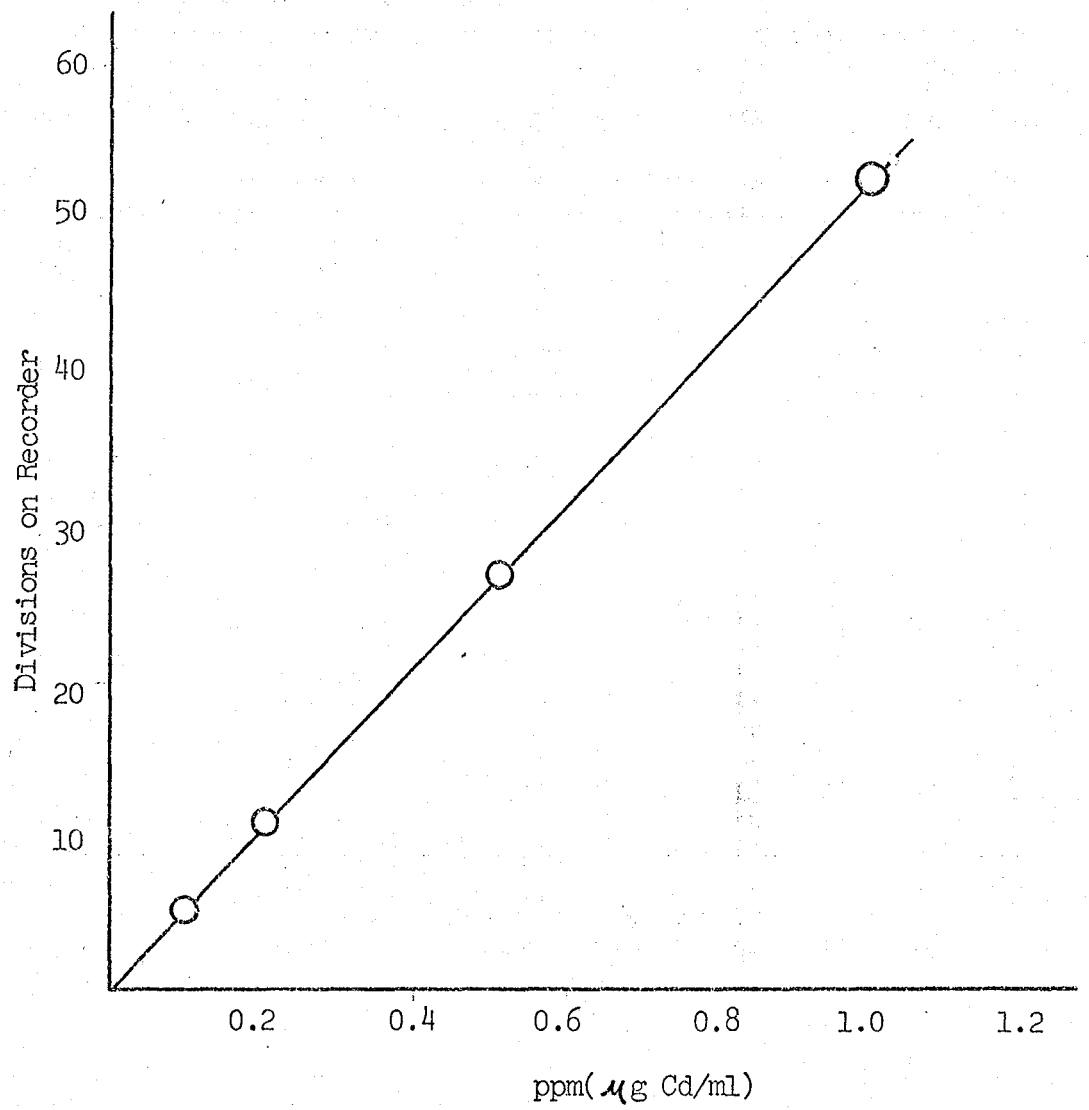

Figure 4:

Standard Curve for Cadmium
Taken from Monitoring Series VII from Dillon Beach

(Range 0.1-1.0 μg Cd/ml)

Table II

Approximate Sizes and the Type of Tissue Used From the Different Specimens for the Monitoring and Accumulation Studies

Genus and Species	Size (inches)		Type of Tissue
	Monitoring	Accumulation	
Pisaster ochreaceus	5-10[a]	5-7[a]	hepatic caeca
Mytilus californianus	2-6 [b]	2 [b]	all except shells
Thais emarginata	1/2 [b]	1/2[b]	all except shells
Pollicipes polymerus	3-6 [c]		all except mantel & stalk cover
Balanus sp	1/2 [d]		all except shell
Kathrina tunicata	2-3 [c]		all except shell
Petrolisthes cinctipes	1/2 [e]		all soft tissue

a Ray span

b Shell length

c Length

d Shell diameter

e Length across the carapace

analysis could be performed.

For the accumulation study only three animals (Pisaster ochraceus, Thais emarginata, Mytilus californianus) were utilized (sizes given in Table II). They were collected live from the Dillon Beach site only and held in large polyethylene bags with a small amount of algae and seawater. On transport to our laboratory they were separately immersed in medium sized polyethylene containers filled with the Instant Ocean solution at room temperature and approximately at pH 8. The Instant Ocean solution was shown to contain non-detectable levels of the three metals by the analytical system employed here. These tanks were then placed in a cold room at 5-6°C under continued aeration. They were held in this manner for about 48-72 hours before beginning the accumulation study. Usually within this period the animals were at least partially purged of mercury, lead, and cadmium.

Procedure for the Accumulation Study:

At the end of the 72 hour period a known amount of one of the metal ion solutions was added to each of three large testing tanks (each of 13 gallon capacity and containing ten gallons of fresh Instant Ocean solution) so that the final concentration of each tank corresponded to the natural ocean-metal concentration (1X) or to some increased multiple of that (5X, 7X, 10X, or 15X). After allowing the solution to mix well for an hour by continuously aerating the tanks, the concentration of the metal in each of the tanks was checked by a.a. spectroscopy. At this point, a few of each of the animals were

taken from their respective holding tanks to serve as controls. Each was wrapped, labeled and sacrificed by freezing. A portion of the remaining animals from the holding tanks were transferred to the respective test tanks in such a way that tank #1 had about 8-10 sea-stars, tank #2 had about 12-14 mussels, and tank #3 contained about 30-40 snails. The animals were withdrawn from the tanks at regular intervals for a period of 24 hours, wrapped, labeled and frozen until they were analyzed.

Tissues Utilized for Analysis:

For both the monitoring and accumulation studies the tissued utilized from the respective animals are as follows:

For Pisaster ochraceus, tissues corresponding to the hepatic caeca were collected (invariably small parts of the gonads and tube feet were also isolated and could not be separated).

For Mytilus californianus, Thais emarginata, Balanus sp., and Kathrina tunicata, all the tissue except the shell or mantle was utilized. For Pollicipes polymerus, the mantle and stalk covering were removed and the remaining inner soft tissues were used for analysis. For Petrolisthes cinctipes, all the soft body tissue (usually only that from the main body cavity) was used. The isolated soft tissues of the larger animals (Pisaster ochreaceus and Mytilus californianus and Kathrina tunicata) were homogenized in a Waring Blender at "high" speed for from one-half to one minute. For the remaining smaller animals, the tissues were minced and then ground with mortar and pestle. The weight of each wet tissue sample was recorded. For

mercury analysis this tissue was used directly; but for lead and cadmium analysis, the wet tissue was dried in an oven at 110°C for a period of twelve hours or until constant weight was achieved. The dried tissue was then stored in a labeled bottle until its analysis could be performed.

Procedure for Mercury Analysis: (Perkin-Elmer Instruction Book 1971)

One gram of homogenized tissue was accurately weighed and placed in a 125 ml Erlenmeyer flask. Thirty ml of concentrated sulfuric acid were slowly added to the flask which was then loosely covered. After waiting for about five minutes, concentrated nitric acid (5 ml) was slowly added to the same flask and it was then loosely recapped. The flask was kept at room temperature for fifteen minutes, then placed on a wrist shaker and simultaneously immersed in a constant temperature water bath for three hours. (shaker setting; low; water temperature, 50-60°C).

The flask was then removed, cooled to room temperature, and its clear contents slowly transferred to a 300 ml B.O.D. bottle containing 50 ml of cold, mercury-free, distilled water. The flask was washed with two 10 ml portions of mercury-free distilled water and each wash added to the B.O.D. bottle. To this clear solution, potassium permanganate crystals (~1 gm) were added, and the flask was heated for one hour in a 50-60°C water bath. The solution turned brown and a froth was formed. When frothing had subsided, more potassium permanganate was added until the purple color persisted.

Hydroxylamine hydrochloride crystals (~1 gm) were slowly added

to the B.O.D. bottle with constant swirling, until the sample turned clear.

Finally, 5 ml of aqueous 10% stannous chloride were added to the B.O.D. bottle, and immediately the aerator probe of the mercury analysis system was inserted into the B.O.D. bottle, and the maximum recorder reading taken.

Procedure for Lead and Cadmium Analysis

About one gram of dry tissue was accurately weighed and put into a 400 ml beaker. To this, 1 ml of 70% perchloric acid and 30 ml of 70% nitric acid were added. The beaker was then placed aside for one hour before heating. After that time the beaker was placed on a hot plate at the #3 setting (90-100°C) until the final volume was reduced to about 1 - 2 ml. This was then diluted with approximately 20 ml of deionized and double distilled water and the pH of the respective solutions was adjusted to 7 for cadmium and to 2.8 for lead analysis with 2.5% NaOH solution. The contents of the beaker were then transferred to a 250 ml separatory funnel. One ml of the appropriate chelating agent and 10 ml of the organic solvent (MIBK) were added.[1] The funnel was shaken for two minutes manually. It was then allowed to stand for a period of five minutes or until all the contents had settled. The aqueous layer was discarded and the organic phase collected

[1] Initially NDDC was used as the chelating agent for lead as well as cadmium, but use of this agent either gave very low or undetectable results for samples for which APDC gave measureable results. Thus APDC became the chelating agent of choice for lead while NDDC proved more satisfactory for cadmium.

in a 10 ml volumetric flask which was then filled to volume with MIBK. This was directly subjected to atomic-absorption analysis by inserting the nebulizer tubing into the volumetric flask and recording the absorption reading. An air-acetylene mixture was used as the source for the flame for both metals.

Sea Water and Instant Ocean Analysis:

To determine the levels of mercury and cadmium in sea water samples and in instant ocean solutions (before and after dosing) a 100 ml aliquot of the sample was subjected to the appropriate analytical procedure by preparing the sample as described on previous pages. On account of the low atomic absorption sensitivity of lead, it was necessary to distill a 1500 ml sample of either sea water or instant ocean solution until about 100 ml remained. This sample was then subjected to lead analysis described by the procedure on previous pages.

Evaluation of the Techniques:

The validity of the experimental procedures used in this investigation was evaluated by adding a known amount of standard metal to homogenized tissue samples of different species for which the tissue metal concentration had already been determined by the regular monitoring analysis and which served as the control. On an average, the percentage recovery for mercury and lead was found to be around 80%, while that for cadmium was shown to be around 90%. The recovery data is presented in Table III.

Table III

Recovery of Mercury, Lead, and Cadmium

Mercury

Name of Species	Control Amount present(µg)	Metal Added(µg)	Total Metal present(g)	Total Metal recovered(µg)	Re- Recovered (%)
Pisaster oc.	0.05	0.7	0.75	0.24	32.0
Mytilus cal.	0.065	0.7	0.765	0.650	85.5
Thais em.	0.07	0.5	0.57	0.490	86.0
Pollicipes pol.	0.14	0.5	0.640	0.478	75.0
Kathrina tun.	0.12	0.5	0.620	0.630	105.0

Lead

Pisaster oc.	17.0	30	47.00	39.68	84.4
Mytilus cal.	6.50	30	36.50	48.72	130.3
Thais em.	18.00	30	48.00	39.41	82.1
Pollicipes pol.	---	30	30.00	25.00	83.3
Kathrina tun.	11.00	30	41.00	32.93	80.3
Balanus sp.	15.50	30	45.50	36.94	81.2

Cadmium

Pisaster oc.	2.65	0.5	3.15	2.82	89.5
Mytilus cal.	3.65	0.5	4.15	3.90	94.00
Thais em.	5.00	0.5	5.50	4.98	90.0
Pollicipes pol.	12.80	0.5	13.30	12.87	96.8
Kathrina tun.	30.00	0.5	30.5	49.52	161.7
Balanus sp.	0.90	0.5	1.30	1.44	110.8

Table IV (continued)

Name of Species	Mercury (ppm)[a]		Lead (ppm)[b]		Cadmium (ppm)[b]	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Petrolisthes cinctipes	XXX	0.91	XXX	XXX	2.0	XXX
Anthopleurea xanthogrammica	XXX	0.04 (0.02-0.06)	XXX	XXX	1.7	XXX
Phargmatopoma californica	XXX	0.03	XXX	XXX	XXX	XXX

Table V

Monitoring Data for the Various Animals Collected from Dillon Beach

Series: II

Date collected: 7-19-73

Name of Species	Mercury (ppm)[a]		Lead (ppm)[b]		Cadmium (ppm)[c]	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Pollicipes polymerus	0.17	0.02	XXX	XXX	5.38	0.74
Balanus sp.	XXX	0.04	XXX	XXX	XXX	XXX
Mytilus californianus	0.21	0.02	5.24	0.59	0.43	0.04
Thais emarginata	0.10	0.03	1.89	0.47	6.98	1.74
Pisaster ochreaceus	0.35 (0.13-0.93)	0.09 (0.03-0.18)	18.20 (10.8-25.6)	XXX	0.25 (0.1 - 0.4)	XXX
Kathrina tunicata	0.18	0.03	19.47	3.69	1.38	0.26
Cucumaria minimata	0.30	0.05	XXX	XXX	XXX	XXX
Petrolisthes cinctipes	XXX	0.93	XXX	XXX	XXX	XXX
Anthopleurea xanthogrammica						
Phargmatopoma californica	XXX	0.03	XXX	XXX	XXX	XXX

Table VI

Monitoring Data for the Various Animals Collected from Dillon Beach

Series: III

Date Collected: 10-3-72

Name of Species	Mercury (ppm)[a]		Lead (ppm)[b]		Cadmium (ppm)[b]	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Pollicipes polymerus	0.69	0.09	2.05	0.28	6.17	0.59
Balanus balonoides sp.	XXX	XXX	XXX	XXX	XXX	XXX
Mytilus californianus	0.34	0.04	5.16	0.61	3.6	0.42
Thais emarginata	XXX	0.06	XXX	XXX	XXX	XXX
Pisaster ochreaceus	0.19	0.03	16.18	2.94	2.39	0.43
Kathrina tunicata	0.53	0.09	10.78	1.88	18.12	3.16
Anthopleurea xanthogrammica	0.41	0.07	14.56	2.42	6.43	1.07

Table VII

Monitoring Data for the Various Animals Collected from Dillon Beach

Series: IV

Date collected: 11-9-1972

Name of Species	Mercury (ppm)[a]		Lead (ppm)[b]		Cadmium (ppm)[b]	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Pollicipes polymerus	0.21	0.02	23.8	2.75	22.27	2.58
Balanus sp.	XXX	0.01	---	---	---	---
Mytilus californianus	0.15	0.03	23.60	4.30	2.68	0.52
Thais emarginata	0.20	0.06	24.40	7.39	23.16	6.5
Pisaster ochreaceus	0.31 (0.30-0.32)	0.077 (0.07-0.08)	27.65	5.02	13.10	2.38
Kathrina tunicata	---	---	14.24	1.98	2.14	0.29
Corallina chilensis	0.21	0.02	11.23	1.23	---	---
Seawater	XXX	0.0005	XXX	---	XXX	0.0016

Table VIII

Monitoring Data for the Various Animals Collected from Dillon Beach

Series: V

Date collected: 12-22-72

Name of Species	Mercury (ppm)[a]		Lead (ppm)[b]		Cadmium (ppm)[b]	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Pollicipes polymerus	0.15	0.03	9.69	1.73	14.93	2.68
Balanus sp.	0.22	0.03	12.90	1.62	0.81	0.10
Mytilus californianus	0.08 (0.05-0.10)	0.017 (0.01-0.02)	10.50	2.50	1.27	0.29
Thais emarginata	0.10	0.03	12.60	3.80	6.19	1.86
Pisaster ochreaceus	0.28 (0.21-0.34)	0.057 (0.055-0.06)	9.20	2.40	1.45	0.37
Kathrina tunicata	0.19	0.04	—	—	2.04	0.41
Corallina chilensis	0.03	0.02	9.38	4.46	2.09	0.99
Seawater	XXX	0.0005	XXX	—	XXX	0.0015

Table IX

Monitoring Data for the Varicus Animals Collected from Dillon Beach

Series: VI

Date collected 2-1-72

Name of species	Mercury (ppm)[c]		Lead (ppm)[d]		Cadmium (ppm)[d]	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Pollicipes polymerus	0.07 (0.03-0.10)	0.013 (0.01-0.02)	—	—	12.82 (12.57-13.35)	2.03 (1.84-2.37)
Balanus sp.	0.12 (0.09-0.16)	0.06 (0.04-0.07)	14.81 (14.5-15.3)	5.80 (4.70-7.0)	1.54 (1.30-1.80)	0.74 (0.61-0.86)
Mytilus californianus	0.17 (0.15-0.19)	0.034 (0.027-0.04)	14.59	2.75	1.36 (0.78-2.12)	0.29 (0.14-0.50)
Thais emarginata	0.14 (0.09-0.19)	0.06 (0.03-0.07)	16.48 (16.18-16.73)	6.35 (5.39-7.57)	2.98 (2.88-3.16)	1.15 (0.96-1.32)
Pisaster ochreaceus	0.26 (0.23-0.32)	0.06 (0.057-0.07)	13.89 (13.22-14.56)	3.69 (3.27-4.12)	5.89 (5.70-6.00)	1.51 (1.32-1.74)
Kathrina tunicata	0.36 (0.18-0.53)	0.07 (0.04-0.09)	13.80 (13.53-14.00)	3.34 (2.43-3.94)	4.31 (4.27-4.36)	1.20 (1.17-1.24)
Corallina chilensis	0.03	0.01	—	—	2.59	1.15
Seawater	XXX	0.0002	XXX	—	XXX	0.0015

Table X

Monitoring Data for the Various Animals Collected from Dillon Beach

Series: VII

Date collected: 4-19-73

Name of Species	Mercury (ppm)[c]		Lead (ppm)[d]		Cadmium (ppm)[d]	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Pollicipes polymerus	0.82 (0.74-0.89)	0.13 (0.11-0.74)	18.90 (18.6-19.3)	2.97	8.40 (7.87-9.05)	1.32 (1.25-1.45)
Balanus sp.	0.15 (0.14-0.17)	0.05 (0.047-0.055)	18.20 (16.5-19.5)	5.97 (5.42-6.40)	8.03 (7.11-9.25)	2.72 (2.33-3.03)
Mytilus californianus	0.72 (0.60-0.95)	0.09 (0.07-0.12)	22.1 (16.6-29.9)	2.75 (2.05-3.75)	6.25 (5.13-6.85)	0.81 (0.78-0.85)
Thais emarginata	0.183 (0.18-0.19)	0.052 (0.047-0.058)	18.9 (18.1-20.2)	5.62 (4.87-6.40)	12.91 (12.04-13.39)	4.09 (3.16-4.99)
Pisaster ochreaceus	0.34 (0.21-0.48)	0.075 (0.07-0.08)	19.7 (19.6-19.8)	3.99 (3.30-4.69)	6.58 (1.43-10.30)	1.27 (0.24-2.05)
Kathrina tunicata	0.27 (0.19-0.40)	0.055 (0.051-0.069)	22.0 (17.7-26.6)	4.77 (3.31-5.57)	10.81 (5.72-13.74)	2.33 (2.04-2.72)
Corallina chilensis	0.35	0.07	19.13	3.72	—	—
Petrolisthes cinctipes	1.94	0.83	19.5	7.4	—	—
Seawater	XXX	0.0003	XXX	0.0016	XXX	0.0015

Table XI

Monitoring Data for the Various Animals Collected from San Francisco Bay

Series: I

Date collected: 9-17-72

Name of Species	Mercury (ppm)[a]		Lead (ppm)[b]		Cadmium (ppm)[b]	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Pollicipes polymerus	0.11	0.03	5.09	1.25	2.53	0.62
Balanus sp.	—	—	5.11	1.34	2.30	0.60
Mytilus californianus	0.09	0.03	—	—	1.02	0.23
Thais emarginata	XXX	XXX	XXX	XXX	XXX	XXX
Pisaster ochreaceus	0.16	0.054 (0.048-0.06)	19.3 (9.5-29.1)	6.0 (3.2-8.8)	1.47 (0.76-1.99)	0.49 (0.27-0.7)
Mopalia sp.	0.08	0.03	3.3	1.4	—	—

a - b - See page

Table XII

Monitoring Data for the Various Animals Collected from San Francisco Bay

Series: II

Date collected: 11-12-72

Name of Species	Mercury (ppm)[c]		Lead (ppm)[d]		Cadmium (ppm)[d]	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Pollicipes polymerus	0.287 (0.283-0.291)	0.038 (0.035-0.041)	31.8	3.94	14.8	1.83
Balanus sp.	XXX	0.04	18.8	XXX	---	---
Mytilus californianus	0.56 (0.55-0.58)	0.094 (0.090-0.097)	19.5	3.2	2.5	0.4
Thais emarginata	XXX	0.07	28.1	8.5	14.51	4.39
Pisaster ochreaceus	0.112 (0.111-0.114)	0.035 (0.034-0.037)	31.9	16.3	1.89 (1.55-2.24)	0.62 (0.46-0.77)
Mopalia sp.	0.05	0.01	XXX	XXX	XXX	XXX

c - g - See page

Table XIII

Monitoring Data for the Various Animals Collected from San Francisco Bay

Series: III

Date collected: 2-11-73

Name of Species	Mercury (ppm)[c]		Lead (ppm)[c]		Cadmium (ppm)[d]	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Pollicipes polymerus	0.27 (0.14-0.39)	0.04 (0.02-0.06)	23.8 (21.9-27.4)	3.7 (3.1-4.3)	4.55 (4.34-4.84)	0.73 (0.69-0.78)
Balanus sp.	0.13 (0.08-0.17)	0.04 (0.03-0.05)	17.24	5.43	5.26	1.68
Mytilus californianus	0.19 (0.15-0.24)	0.028 (0.024-0.031)	19.5 (19.0-19.8)	2.9 (2.6-3.0)	0.65 (0.19-1.57)	0.025 (0.02-0.03)
Thais emarginata	0.172 (0.167-0.177)	0.05 (0.049-0.052)	9.47	2.78	1.60	0.47
Pisaster ochreaceus	0.08	0.03	14.6 (14.3-14.9)	4.3 (3.4-5.3)	1.16 (1.11-1.21)	0.33 (0.24-0.43)
Mopalia sp.	0.15 (0.14-0.17)	0.04 (0.03-0.06)	9.97	3.50 (3.0-4.0)	7.64 (7.52-7.76)	2.22 (1.94-2.50)
Seawater	XXX	0.0005	XXX	—	XXX	0.0017

Table XIV

Monitoring Data for the Various Animals Collected from San Francisco Bay

Series: IV

Date collected: 4-8-73

Name of Species	Mercury (ppm)[c]		Lead (ppm)[d]		Cadmium (ppm)[d]	
	Dry wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Pollicipes polymerus	0.43 (0.32-0.54)	0.08 (0.06-0.10)	11.23 (10.98-11.72)	2.03 (1.76-2.24)	13.36 (11.79-14.65)	2.55 (2.25-2.80)
Balanus sp.	0.09	0.03	17.71 (17.62-17.77)	8.37 (8.36-8.38)	8.45 (7.26-10.24)	3.96 (3.37-4.76)
Mytilus californianus	0.53 (0.32-0.95)	0.07 (0.04-0.11)	11.53 (11.26-16.92)	2.76 (2.21-4.72)	4.22 (0.92-6.01)	0.82 (0.11-1.69)
Thais emarginata	0.24 (0.19-0.29)	0.08 (0.06-0.10)	16.80 (16.10-17.60)	5.7 (5.48-6.00)	4.83 (4.71-4.95)	1.64 (1.60-1.69)
Pisaster ochreaceus	0.10	0.03	2.45	0.56	11.96	2.71
Mopalia sp.	0.42 (0.41-0.44)	0.10 (0.09-0.11)	10.98 (10-96-11.04)	3.69 (3.46-3.84)	3.77 (3.11-4.20)	1.07 (1.03-1.10)
Seawater	XXX	0.0005	XXX	XXX	XXX	0.0016

a. For the monitoring of mercury for all specimens, the value represents a determination of just one (i.e. non-pooled) sample, unless the value represents an average of two or more specimens where the range is given in brackets.

b. For the monitoring of lead and cadmium, at least ten specimens of each of Thais em., Balanus sp., Pollicipes pol., Petrolisthes cin., Cucumaria min., and Corallina ch. were pooled. As such the reported level represents an "average" tissue level for each pooled specimen. For Pisaster och., Mytilus cal., Anthopleurea xan., Compound Ascidian, Kathrina tun., and Mopalia sp., the reported level represents a tissue level from a single specimen unless it is an average of two or more separate specimens where the range given in brackets.

c. The tissue level represents an average of three separate analyses on individual specimens where the range is given in brackets.

d. For Thais em., Balanus sp., Pollicipes pol., Petrolisthes cin., Cucumaria min., and Corallina ch., at least three specimens were pooled for each determination. The average value represents the mean of three such pooled values, whose range is given in brackets. For Pisaster och., Mytilus cal., Anthopleurea xan., Compound Ascidian, Kathrina tun., and Mopalia sp., a minimum of three individual specimens of each species were analyzed; their average value is given with the range in brackets.

e. (---) represents a non-detectable level

f. XXX means either that the sample was not collected, or that an insufficient amount was available for analysis for all three metals.

g. NDDC was used as chelating agent.

Procedure for Protein Analysis (Lowry, 1951):

Chemicals: (All reagents and chemicals used were of reagent grade)

A. 2% Na_2CO_3 in 0.1N NaOH

B. 0.5% $CuSO_4 \cdot 5H_2O$ in 1% aqueous sodium tartrate (freshly prepared)

C. 50 ml A + 1.0 ml B

D. 50 ml aqueous 2% Na_2CO_3 + 1 ml B

E. Folin phenol reagent (Make 1:1 aqueous dilution of stock phenol reagent.)

Procedure:

For the determination of percent total protein content, wet homogenized tissue from each of the different specimens was used. The tissues used were the same as the monitoring study and each was homogenized in a manner similar to one used for the monitoring analysis. The total amount of water in the tissue was determined by drying a large aliquot of the tissue to constant weight in the usual manner. The dehydration factor allowed calculation of the percent protein content on a dry weight basis as reported in Table XVIII. About 0.5 g of the homogenized, wet tissue was weighed and solubilized in a beaker containing about 50 ml of triple distilled water by mixing with a stirring rod for about five minutes. The solution was then placed aside for about 30 minutes. The supernatant liquid was separated, its volume determined, and was then used for the determination of soluble proteins. The precipitate was set aside to be used for the determination of the insoluble protein fraction. For the soluble

protein fraction, 0.5 ml of the liquid was pipetted to an absorption tube to which 4.0 ml of reagent C was added. The contents of the tube were thoroughly mixed and set aside for 10 minutes. At this time 0.5 ml of reagent E was added and immediately mixed on a tube shaker. The tube was allowed to stand thirty minutes, after which its optical density was determined at 640 m in a Weston colorimeter (Model #834).

For the insoluble protein fraction, the residue was dissolved in 0.4 ml of 1.0N NaOH. Slight heating was necessary to dissolve the precipitate. To this, 4.0 ml of reagent D was added and remaining procedure was the same as that for the soluble protein determination described above.

Total protein content on a wet weight basis was calculated by comparing the above results with a standard Beer's law plot determined using standard solutions of bovine albumin. The total protein content was then converted to dry weight basis and also reported. (Table XVIII)

These determinations were carried out in triplicate. The specimens were collected at the Dillon Beach site on May 13, 1973.

DISCUSSION OF RESULTS

Introduction to the Monitoring Study:

The monitoring data for the various animals collected from Dillon Beach (seven collections from June, 1972 to April, 1973) and Land's End (four collections from September, 1972 to April, 1973) is presented in Tables IV to XIV.

In the experimental procedure, mercury was analyzed on the wet-weight basis while lead and cadmium were determined on a dry-weight basis. The experimental wet tissue levels for mercury were converted to dry weight values by mathematical conversion for each specimen on a basis of the amount of water lost on drying to constant dry-tissue weight. Similarly, levels of dry tissues for lead and cadmium were converted to wet-weight values in the same manners.

Perusal of the data in Tables IV through XIV shows a considerable variation in the mean tissue levels of the metals for a given set of specimens of one species from one sampling date to the next regardless of whether it was collected during the dry (June to October) or wet (November to April) seasonal sampling period. Not only is there variation between sampling dates within a given season (dry or wet), but also it can be seen that there is considerable variation between individual samples of the same species collected on the same date and at the same site (for example, note the ranges presented in Tables IX or X). Thus, any attempt to establish a "base line" for the three metals considered here, even with recognition of seasonal changes

which will be shown and discussed later, must be done only in light of the fact that individual variation within a species is a very appreciable factor at any given time of the year. Examination of the data presented by Graham (1972) and Schwimer (1973) tends to reinforce this observation, while that from Bryan (1973) definitely demonstrates both individual and seasonal variation.

Literature:

Mercury levels for the animals under investigation in this study taken from both sites ranged from 0.01-0.93 ppm on the wet-weight basis. The highest levels (0.83-0.93 ppm) were found in Petrolisthes cinctipes, a scavenger and perhaps the most "mobile" of the animals examined here.

Swanson (1973) examining mercury levels in the various tissues of the purple shore crab, Hemigrapsus nudus, collected at Dillon Beach during the Spring of 1973 has found mean values on a wet-tissue basis of 0.20 ppm in carapace, 0.43 ppm in the gills, 0.09 ppm in the hepato-pancreas, and 0.14 ppm in the stomach. Tissue to water concentration factors have also been given and are respectively 833, 1796, 390 and 609.

Burch (1972) has reported the range of mercury levels on a wet-tissue basis in various organs of the striped bass, Roccus saxatilis, taken from a site on the Sacramento River near Rio Vista around March-April 1971. The mean levels averaged as low as 0.11 ppm in the bile to as high as 3.43 in the liver. However, in contrast to the immobile animals examined here, striped bass migrate from Delta waters to

sandy beaches as far away as those of Marin and San Mateo counties. Considering the differences in mobility, in food sources or trophic order, and in other factors, the differences in mercury levels in animals of the mussel bed community and those in indigenous fish may not be all that great.

Further monitoring studies of mercury in fish, in general, taken from eastern U.S. fresh waters or from the Atlantic Ocean, are given by Zitko, et al. (1971), Scott and Armstrong (1972), Kamps, et al (1972), Rivers, et al (1972), and Windom, et al (1973). Similar studies for cadmium are given by Lovett, et al (1972) and Windom, et al (1973). Klein and Goldberg (1970) have reported mercury levels in sediment and in selected inbertebrates taken from the Palos Verdes-La Jolla area of southern California. He reports that "variations in mercury levels between species were the same essentially as those within a species, that mercury levels in organisms near the (sewer) outfall were comparable to those far removed but that concentrations in sediments were higher close to a sewer outfall and lower farther away". On a dry-tissue weight basis, the highest level was 21 ppm in a cowry while the lowest was 0.4 ppm in a sea cucumber. That author's range in values is somewhat higher than those presented herein, although his conclusions are essentially the same as those reported here.

On the dry-tissue weight basis, mean lead levels in this study from both the sites ranged from 1.0-31.9 ppm while mean cadmium levels varied from 0.3-23.2 ppm. Graham (1972) has reported the dry-tissue weight levels of lead, cadmium and other metals in several intertidal

mollusks collected at an unstated time of year (probably the summer of 1971). The average amounts of lead and cadmium found by Graham in Mytilus californianus and Thais emarginata at different sites between San Francisco and Los Angeles (most are located between San Francisco and Monterey Bays) are given in Table XV along with averages and ranges of corresponding data collected during the course of this study.

Regardless of the fact that Graham collected his animals from sites near sewage outfalls or within yacht harbors which are possibly more polluted than the two sites considered in this study, the metallic levels found in the same species from both studies are in close proximity of each other.

Schwimer (1973) has reported dry-tissue weight levels of many metals including lead and cadmium in specic tissues of Pisaster brevispinus (a soft-bottom, quiet-water dwelling subspecies of Pisaster) as well as two other invertebrates collected in the Monterey Bay area at an uspecified time of year (probably in the summer of 1972). That author's results and those collected in the course of this study are compared in Table XVI.

Brooks and Rumsby (1965), collecting specimens in New Zealand waters, have reported the dry tissue levels of lead, cadmium and other metals (as well as the tissue to water concentration factors) in a scallop (Ostrea sinuata), oyster (Pecten noval-zelandiae), and mussel (Mytilus edulis aoteanus). The average values for the total soft tissues of each are respectively: 16(5,300) and 249(2,260,000) ppm; 10(3,300) and 35(318,000) ppm; and 12(4000) and 10(100,000) ppm. With the exception of cadmium in the scallop, the authors tissue-metal

Table XV

Comparison of Data from Graham's and This Study

Source of information	Species	Site	Lead ppm	Cadmium ppm
Graham's Study	Mytilus calif.	Half Moon Bay	23.4±3.0[a]	4.9±0.6[a]
	" "	Carmel Bay	2.2	2.0±0.0
	" "	White's Point	7.8±1.3	2.0±0.0
	Thais emarginata	Fisherman's Wharf, Monterey	2.2	13.5±0.1
This Study	Mytilus calif.	Dillon Beach	12.00[b] (2.7-23.6)	2.4[b] (0.4-6.3)
	" "	Land's End	16.8 (11.5-19.5)	2.1 (0.7-4.2)
	Thais emarginata	Dillon Beach	14.85 (1.9-24.4)	9.1 (2.4-23.2)
	" "	Land's End	18.10 (9.5-28.1)	6.9 (1.6-14.5)

a. Data represents means and standard deviation of analysis of 3 separate aliquots.

b. Data represents mean with observed range of values

Table XVI

Comparison of Date From Schwimer's and This Study

Schwimer's study	*Pisaster brevispinus* hepatic caecum	Fisherman's Wharf, Monterey	non-detectable value	7.1 ± 2.1
		Monterey sewage outfall	non-detectable value	46.3 ±20.6
This study	*Pisaster ochreaceus* hepatic caecum	Dillon Beach	15.6 (4.4-27.65)	5.6 (0.25-9.4)
		Land's End	17.1 (2.45-31.9)	4.1 (1.16-11.96)

results compare closely with those presented here for other invertebrates. They have also reported the metal levels in the shells and all individual internal organs for each animal. The seawater concentrations of lead and cadmium are "estimated" literature values and are assumed to be 3.0 and 0.11 ppb thus the concentration factors especially for cadmium are questionable. No date(s) are given for the collection of specimens and no distinction is made between the two collecting sites utilized.

The monitoring and accumulation of trace metals including lead and cadmium in estuarine mollusks including the Pacific oyster (Crassostrea gigas) collected from Burley Lagoon, Washington, over a period of two years, has been reported by Pringle, et al. (1968). On a wet-tissue basis, the levels of cadmium (1.17 ppm) are comparable to those here, but those for lead (< 0.2 ppm) are approximately an order of magnitude lower. Little if any seasonal variation was apparent.

Pyramiding with Consumer Order:

The results of this monitoring study may be seen by examination of the data presented in Table XVII which represents for the respective collecting areas the mean and range of seven and four sets of specimens. In no case for either mercury, lead, or cadmium does the tissue of the common sea star (on either a wet- or dry-weight basis) clearly contain the highest level of the metals as might be expected for this tertiary consumer. Perhaps the purple rock snail, a secondary consumer, is the only animal examined that exhibits wet-tissue levels that are

Table XVII

Averages (and Ranges) of Metal Levels in the Mussel Bed Community at Dillon Beach

Dillon Beach	Mercury (ppm)		Lead (ppm)		Cadmium (ppm)	
Name	Dry wt.	Wet. wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Pollicipes polymerus	0.35 (0.07-0.82)	0.04 (0.01-0.13)	11.0 (1.0-18.9)	1.93 (0.28-2.97)	10.5 (3.5-22.3)	1.32 (0.59-2.68)
Balanus sp.	0.16 (0.12-0.22)	0.04 (0.01-0.06)	15.3 (1.7-18.2)	4.46 (1.62-5.97)	3.4 (0.8-8.0)	1.18 (0.1-2.72)
Mytilus californianus	0.28 (0.08-0.72)	0.05 (0.02-0.14)	12.2 (2.7-22.1)	2.00 (0.59-4.3)	2.4 (0.4-6.3)	0.39 (0.04-0.81)
Thais emarginata	0.15 (0.10-0.20)	0.05 (0.03-0.62)	14.8 (1.9-24.4)	4.72 (0.47-7.39)	9.1 (2.4-23.2)	3.08 (1.15-6.50)
Pisaster ochreaceus	0.28 (0.19-0.35)	0.06 (0.03-0.09)	15.6 (4.4-27.7)	3.48 (2.4-3.99)	5.6 (0.3-13.1)	1.19 (0.37-2.38)
Kathrina tunicata	0.30 (0.18-0.53)	0.06 (0.03-0.1)	13.9 (3.6-22.0)	3.13 (1.88-4.77)	5.7 (1.4-18.1)	1.27 (0.26-3.16
Corallina chilensis	0.15 (0.03-0.35)	0.03 (0.01-0.07)	13.2 (9.4-19.1)	3.13 (1.23-4.46)	1.7 (0.5-2.6)	1.07 (0.99-1.15)
Petrolisthes cin	1.94	0.89 (0.83-0.93	19.5 (one set)	7.4	2 (one set)	-----

Table XVII

Averages (and Ranges) of Metal Levels in the Mussel Bed Community at Land's End

Land's End	Mercury (ppm)		Lead (ppm)		Cadmium (ppm)	
Name	Dry Wt.	Wet wt.	Dry wt.	Wet wt.	Dry wt.	Wet wt.
Pollicipes polymerus	0.27 (0.11-0.43)	0.05 (0.03-0.08)	17.9 (5.1-31.8)	2.73 (1.25-3.94)	8.8 (2.5-14.8)	1.43 (0.62-2.55)
Balanus sp	0.11 (0.09-0.13)	0.04 (0.03-0.04)	14.7 (5.1-18.8)	5.04 (1.34-8.37)	5.3 (2.3-8.5)	2.08 (0.6-3.96)
Mytilus californianus	0.34 (0.09-0.56)	0.05 (0.03-0.09)	16.8 (11.5-19.5)	2.95 (2.76-3.2)	2.1 (0.7-4.2)	0.36 (0.025-0.82)
Thais emarginata	0.20 (0.17-0.24)	0.07 (0.05-0.08)	18.1 (9.5-28.1)	5.66 (2.78-8.5)	6.9 (1.6-14.5)	2.13 (0.47-4.39)
Pisaster ochreaceus	0.11 (0.08-1.6)	0.04 (0.03-0.05)	17.0 (14.0-31.9)	6.8 (0.56-16.3)	4.1 (1.2-12.0)	1.03 (0.33-2.71)
Mopalia sp	0.17 (0.05-0.42)	0.05 (0.01-0.05)	8.05 (3.3-10.9)	2.86 (1.4-3.69)	5.7 (3.8-7.6)	1.64 (1.07-2.22)

consistently high (but not necessarily the highest in a given set). With the exception of the procelain crab and only in regard to one metal (mercury) it appears that all the animals examined contain roughly and variably the same levels of the three metals. Indeed, it appears that for all the species examined here, it is not the trophic level that determines tissue metal, rather it is the composition of the environment in which the animal is immersed. Apparently, unless an animal can be captured while it is actively feeding (as will later be discussed for "feeding" sea stars), tissue metal levels are probably most indicative of a given animal's ability to achieve a steady state (absorption-excretion) equilibrium with his marine environment. Such equilibria will later be examined in the discussion of the accumulation of the three metals in the sea star, snail, and mussel. At least with regard to trophic order and tissue metal level, Schwimer (1973) has shown that various metals including lead and cadmium are not concentrated between trophic levels for Olivella biplicata (a herbivorous snail), Polinices lewisii (a large, predatory snail), and Pisaster brevispinus (a predatory sea star) collected in the Monterey Bay area - a consumer chain found in subtidal waters or on sandy or muddy tidal flats. The author also points out that certain unusually high levels of particular metals in a given speciman are probably a consequence of pollution of that particular collecting site. Thus it would appear that for the invertebrates examined here, the tissue metals levels presented in Table XVII indicate that pyramiding is not operative through the trophic levels of the mussel bed consumer chain - at least on the wet or dry tissue weight basis.

Since transition series and certain other metal ions are known to complex to enzymes as activators and/or as inhibitors, it was felt that perhaps tissue levels of the three metals considered here could be better correlated with tissue protein content. Percent total protein content of the species of interest based on their wet tissue weight are presented in Table XVIII. Also included therein are the calculated percentages based on dry tissue weight obtained by drying a portion of wet tissue homogenate under the usual conditions to constant weight. Specimen sizes and the tissues utilized for determination by the Lowry Method (see the experimental chapter) are consistent with those for the monitoring study given in Table II. The protein content values on a dry weight basis determined for the animals in this study correlate reasonably well with those of Geise (1966) presented in Table XIX for the various organs of some of the same animals. Individual values represent the averages of all values obtainable for each species. Giese also points out that age, sex, season, food availability, etc., do play a large role in determining the varying amounts of protein, lipid and carbohydrate as well as organ size in marine invertebrates. As such, since protein content was not investigated during the course of the monitoring study, but only for one sampling (May 19, 1973, for which the metal levels were not determined), extreme caution must be exercised in the following correlations.

On both the wet and dry basis, the porcelain crab was found to have the highest percent protein content (Table XVIII) and clearly the highest tissue mercury content (Table XVII). Unfortunately, there is insufficient data for lead and cadmium levels in this animal and

Table XVIII

Total Protein Content of Animals in This Study

Species	% Protein on Wet Wt. Basis	% Protein on Dry Wt. Basis
Petrolisthes cinctipes	24.0	56.5
Thais emarginata	13.4	33.0
Pisaster ochreacus	7.4	43.3
Mytilus californianus	3.2	25.0
Pollicipes polymerus	3.4	22.0
Kathrina tunicata	5.0	25.0
Mopalia sp.	5.4	22.0
Balanus sp.	7.5	23.0

Table XIX

Total Protein Content in Different Organs from Geise (1966)

Species	Organ	% Protein on Dry Wt. Basis
Pisaster ochreaceus	Body Wall	14.1
	Body Fluid	0.02
	Gut	43.7
	Gonald	37.8
	Ceca	28.3
Mytilus californianus	Foot	66.7
	Mantle	58.7
	Viscera	54.2
	Testis	57.1
	Ovary	50.7
Kathrina tunicata	Foot	42.5 ± 2.2
	Mantle	36.2
	Digestive gland	26.3
	Testis	44.0 ± 1.8
Mopalia hindsii	Foot	49.0 ± 6.3
	Mantle	38.6 ± 7.2
	Digestive gland	24.0 ± 3.4
	Ovary	37.6 ± 2.9
	Testis	50.4 ± 6.8
Petrolisthes cinctipes	Whole	29.4

further correlations are not possible.

On the wet basis (probably the most meaningful basis), the next highest protein content was found in the purple rock snail. As stated previously, each of its wet tissue metal levels is consistently high when compared to the remaining animals examined at either of the two collecting sites (Table XVII). The remaining animals, including the sea star which sits at the apex of this consumer chain, are seen to have the lowest "wet" percent protein content - all approximately of the same value. Not surprisingly the remaining animals also have roughly and variably about the same tissue metal levels.

Site and/or Seasonal Tissue-Metal Level Differentiation:

At the onset of the project, it was felt that the tissue metal levels found in samples collected from the Golden Gate would be higher than those found from Dillon Beach. This was postulated on the basis that San Francisco Bay waters receive vastly more urban and industrial discharge than Dillon Beach waters. On experimentation, this was found not to be the case. The levels of metals in seawater samples and in the tissues of the various animals collected from the two sites were found to be quite close to each other. (Tables IV through XIV and XVII.) The lead and cadmium tissue values obtained by Graham (1972) and by Schwimer (1973) for specimens collected along the immediate coastal area correlate closely with the observations reported here. (Table XV and XVI). This may seem to indicate that in spite of the fact that one area may be thought to be more polluted than the other, the levels of metals accumulated by marine invertebrates at these different sites

remain fairly constant for any one season of the year along the central California coast. It might be said that effective mixing of sea water occurs along these coastal areas at least from Dillon Beach to Monterey Bay. Should similar offshore currents pass through all northeast Pacific waters, then one might expect that virtually the same levels of these metals could be found in whatever "immobile" (and possibly mobile) species that might be investigated from Alaska to Mexico.

As can be seen in Table XVII, there is little difference in the average tissue metal levels for the total monitoring period when the two collecting sites are compared. However, if the data for each site is averaged for its "dry" season and for its "wet" season, then an interesting result is observed. The monthly rainfall data for appropriate sites along the central California coast and the Sacramento and San Joaquin Valleys is presented in Table XX. This data is available from the publication, Climatological Data, by the Environmental Data Service, National Oceanic and Atmospheric Administration, U.S. Department of Commerce. It can be seen that rainfall for the winter of 1971-72 began in earnest in November and continued sporadically through April although the total for that winter was substantially below normal statewide. The summer months of 1972 were typically dry. But heavy rains then began falling in late October and November and continued on through March of 1973 resulting in a winter that was abnormally high in total precipitation. On this basis then it was felt that monitoring data collected for the months June through October, 1972 would be indicative of a "dry" season, while that collected from November, 1972 through April, 1973, would correspond to a "wet" season. The average tissue metal levels calculated for the dry and wet seasons for

each of the two sites are presented in Table XXI and XXII. These tables indicate that the mercury levels in the animals from each site remain fairly close to each other during both the dry and wet seasons, but that there is a considerable difference in the lead and cadmium levels when the seasons are compared for each site.

It is also of note that the dry and wet seasons metal levels from the Dillon Beach site are almost identical to those from the Land's End site just as was demonstrated by the overall levels presented for the two sites in Table XVII. The large increases observed for lead and cadmium levels during the rainy period amplify the fact that it is necessary to qualify any monitoring study on a seasonal basis. Thus in attempting to define a base line for metals in invertebrates, the data of Graham (1972) and of Schwimer (1973) cannot be evaluated since the extent of the collection period and especially the dates of collection are not stated. On the other hand, the data of Bryan (1973) for two scallops off the English coast, which encompasses two and one-half years of monitoring, is of great value. Bryan has been able to correlate a considerable amount of data that definitely demonstrate seasonal variation of many trace metals including lead and cadmium. He finds that although there is considerable variation between individual animals of the same species at any one time, the mean concentrations were found to be highest in the autumn and winter months. Bryan proposes the following explainations for his results on a basis of: a) reproductive cycle, that even though the relative masses of the internal organs (especially gonads, digestive gland and kidneys) change seasonally

Table XX

Rainfall Data for Monitoring Period

Date	Shasta	Ft. Ross	Sacramento WSDC	S.F.	Stockton	Yosemite	Fresno	Bakersfield	Dillon Beach PMS
Sept. 1971	0.74	0.18	T	0.22	0.19	1.04	0.04	0.02	XXX
Oct. "	0.79	1.11	.13	0.13	0.28	1.13	0.03	0.09	XXX
Nov. "	7.63	3.60	.87	1.66	0.81	6.26	0.65	0.12	XXX
Dec. "	7.02	7.30	4.05	4.42	3.82	11.35	2.56	1.17	XXX
Annual 1971	40.56	29.12	9.78	13.73	10.61	33.63	6.99	5.77	XXX
Jan. 1972	6.86	4.61	1.07	1.24	0.69	2.04	0.37	T	XXX
Feb. "	3.65	2.35	1.15	1.50	0.70	1.34	0.67	0.27	XXX
Mar. "	5.36	2.38	0.37	0.29	0.07	0.01	0.00	T	XXX
Apr. "	5.32	2.35	1.27	0.99	0.57	5.86	0.27	0.08	XXX
May "	2.58	0.24	0.34	0.00	0.11	0.22	0.15	0.02	XXX
June "	1.56	0.26	0.15	0.14	0.15	1.29	0.60	1.11	XXX
July "	0.00	0.01	0.00	0.00	0.00	0.00	T	T	XXX
Aug. "	0.08	0.25	0.00	0.04	0.00	1.08	0.00	T	XXX
Sept. "	1.42	1.72	0.99	0.80	0.66	1.97	0.29	0.02	0.65
Oct. "	4.52	3.89	1.70	4.87	0.74	1.49	0.22	0.54	0.70
Nov. "	13.36	8.02	5.08	5.97	6.22	4.48	3.50	1.55	0.75
Dec. "	6.58	4.72	2.25	3.06	2.38	4.30	1.40	0.66	0.39
Annual 1972	51.29	30.80	14.37	18.90	12.29	24.08	7.47	4.25	

Table XX (continued)

Date		Shasta	Ft. Ross	Sacramento WSDC	S.F.	Stockton	Yosemite	Fresno	Bakersfield	Dillon Beach PMS
Jan.	1973	18.96	15.52	7.29	9.26	6.31	6.10	1.91	2.07	XXX
Feb.	"	13.88	6.92	6.47	6.29	4.19	9.57	3.69	0.49	XXX
Mar.	"	8.41	5.64	2.89	2.44	3.18	4.59	2.84	2.49	XXX
Apr.	"			0.41		.23		.09	.18	XXX
May	"			.060		.04		T	T	XXX
				Yearly Rainfall						
1971		40.56	29.12	9.78	13.73	10.61	33.63	6.99	5.77	XXX
1972		51.29	30.80	14.37	18.90	12.29	24.08	7.47	4.25	XXX

T = trace amount of none detectable

Table XXI

Tissue Metal Level (in ppm) and Sea Water Levels (in ppb) for the Dry and Wet Seasons at Dillon Beach

Animal	Average Metal Level for Dry Months			Average Metal Level for Wet Months		
	June-October			November - April		
	Mercury (Wet)	Lead (Dry)	Cadmium (Dry)	Mercury (Wet)	Lead (Dry)	Cadmium (Dry)
Pollicipes polymerus	0.04 (0.02-0.09)	1.5 (1.0-2.05)	5.0 (3.5-6.17)	0.04 (0.01-0.13)	17.4 (9.7-23.8)	17.3 (8.4-22.3)
Balanus sp	0.05 (0.04-0.06)	1.7 (single)	3.2 (single)	0.04 (0.01-0.06)	15.3 (12.9-18.2)	3.4 (0.81-9.03)
Mytilus californianus	0.07 (0.02-0.14)	4.3 (2.7-5.24)	1.8 (0.43-3.6)	0.04 (0.01-0.09)	17.6 (10.5-23.6)	2.9 (1.27-6.25)
Thais emarginata	0.25 (0.03-0.62)	1.9 (single)	4.7 (2.4-6.98)	0.04 (0.03-0.06)	18.1 (12.6-24.4)	11.3 (3.0-23.16)
Pisaster ochreaceus	0.05 (0.03-0.09)	12.9 (4.4-18.2)	4.00 (0.25-9.4)	0.07 (0.05-0.07)	17.6 (4.0-27.6)	6.8 (1.45-13.1)
Kathrina tunicata	0.07 (0.03-0.1)	11.3 (3.6-19.4)	7.0 (1.38-18.12)	0.06 (0.04-0.07)	16.7 (13.9-22)	4.8 (2.04-10.81)
Corallina chilensis	0.05 (single)	---[a]	0.5[a]	0.03 (0.01-0.07)	13.2 (9.38-19.13)	2.34 (2.09-2.59)
Petrolisthes cinctips	0.92 (0.91-0.93)	XXX	2.0[a]	0.83[a]	19.5[a]	XXX
Sea Water	XXX	XXX	XXX	0.4 (0.2-0.5)	1.6[a]	1.55 (1.5-1.6)

a = only one animal studied

Table XXII

Tissue Metal Levels (in ppm) and Sea Water Levels (in ppb) for the Dry and Wet Seasons at Land's End. San Francisco Bay

Animal	Average Metal Level for Dry Months June-October			Average Metal Level for Wet Months November-April		
	Mercury (Wet)	Lead (Dry)	Cadmium(Dry)	Mercury (Wet)	Lead (Dry)	Cadmium (Dry)
Pollicipes polymerus	0.03	5.09	2.53	0.05 (0.04-0.08)	22.3 (11.23-31.8)	10.9 (4.55-14.8)
Balanus sp.	—	5.11	2.30	0.04 (0.03-0.04)	17.9 (17.2-18.8)	6.8 (5.26-8.45)
Mytilus californianus	0.09	—	1.02	0.06 (0.028-0.094)	16.8 (11.53-19.50)	2.45 (0.65-4.22)
Thais emarginata	XXX	XXX	XXX	0.06 (0.05-0.08)	13.3 (9.47-28.1)	6.9 (1.60-14.51)
Pisaster ochreaceus	0.16	19.3	1.47	0.03 (0.03-0.035)	16.3 (2.45-31.9)	5.0 (1.16-11.96)
Mopalia sp.	0.08	3.3	—	0.05 (0.01-0.1)	10.5 (9.97-10.98)	5.7 (3.77-7.64)
Sea Water	XXX	XXX	XXX	0.5 (0.5)	—	1.6 (1.6-1.7)

the mean metal concentrations were not significantly different from month to month; b) temperature, although a decrease in metal levels occurred during the warmer seawater months ($\backsim$15°C for July through September) with the probable increased ability of the animals to excrete metals, many minimum tissue levels were recorded during April and May when the water temperature was still as low ($\backsim$8°C) as it was through the winter; c) land drainage, even though the increased mean flow rate of large rivers near the collecting site can be correlated with an increase in tissue levels for most of the metals examined, some metals show negative correlation with river input; d) the availability of food, there is a definite inverse correlation between the phytoplankton productivity of late spring and summer and the tissue metal concentrations. Thus Bryan concludes that "although changes in temperature and in the input of trace metals from rivers may be involved in producing seasonal changes in the levels of trace metals in scallops, it is thought that changes in phytoplankton productivity are more important".

With regards to the data presented in Tables XXI and XXII, it is apparent that although there are undoubtedly many factors responsible for the change in tissue metal levels from the dry to wet season -- the overriding factor being rainfall. The external environment is exposed to lead from that in auto exhaust deposited on the roadway and that as a pigment in paint covering almost all of man's construction works. Cadmium, meanwhile, is distributed as a pigment in both paint and plastic, as a contaminant in superphosphate fertilizers, and to a small extent as a turf fungicide. Thus rain "mobilizes" lead and cadmium, washing

it from streets and building exteriors of the urban Bay Area and from the highways, towns and agricultural fields of the Great Valley - all eventually funnel through the Golden Gate.

The lack of change found for mercury can be rationalized on the basis that its greatest contribution to the environment is through industrial discharge which in this case is not only localized about and directed into San Francisco Bay, but also is probably maintained at a constant rate throughout the year. The fact that mercurial derivatives are used as seed fungicides is probably not important here since the amount used is limited and small, and since mercury is strongly bound by soil which prevents its leaching. Thus the levels of mercury should not greatly fluctuate with rainfall in this situation.

Thus, in contrast to Bryan's conclusions (1973), "land drainage" appears to be the most important factor here, although unfortunately the seawater concentrations for the three metals were not monitored during the "dry" season for comparison to the "wet". (Tables XXI and XXII). Phytoplankton productivity in these waters should be on the decrease in September and October; however, metal levels for these monitoring dates are still low in general. Also since there is so much variation when comparing the metal levels for any one date with any other (Tables IV through XIV), it would seem that phytoplankton productivity cannot be the major factor here. No data was collected during this study for seawater temperature variation or for reproductive and other organ mass fluctuations, therefore, the importance of these factors cannot be assessed. However, it is interesting to note here that

during the winter months, central California ocean temperatures do drop from ∽15°C to ∽10°C, and with that the sea star and purple snail, as well as others, are seen to cease feeding and begin reproductive activities. At least in the case of the sea star, commencement of active feeding at Dillon Beach was not observed until May, 1973 - thus the winter, 1972 through spring, 1973, monitoring data should at least reflect tissue levels for specimens that were not feeding and whose basal metabolic rate was comparatively low. It is noteable then that mean lead and cadmium levels for all the animals monitored from November through April were relatively high compared to earlier monitorings. However, the same trend if rationalized on a basis of water temperature, reproductive cycle, active feeding, or planktonic productivity does not hold true for mercury levels. Thus the major factor among those considered here determining the mercury, lead and cadmium levels in invertebrates along our immediate coastline must be rainfall and the subsequent runoff.

Environmental Concentration Factors:

Finally, concentration or enrichment factors (the ratio of tissue to water levels of a metal) can be calculated from the data presented in Table XXI for the Dillon Beach specimens collected from November through April. These are presented in Table XXIII. For convenience of literature comparison, mercury factors are based on wet-tissue weight and lead and cadmium factors are based on dry-tissue weight.

Apparently all the animals are about equally proficient in accumulating lead, while the porcelain crab and goose barnacle appear to ac-

Table XXIII

Concentration Factors of Tissues of Various Animals

Species	Hg (wet)	Pb (dry)	Cd (dry)
Pollicipes polymerus	100	10875	11160
Balanus sp	100	9625	2192
Mytilus californianus	100	11000	1870
Thais emarginata	100	11310	7290
Pisaster ochreaceus	175	11000	4380
Kathrina tunicata	150	10440	3095
Corillina chilensis	75	8250	1510
Petrolisthes cinctipes	2075	12175	XXX

cumulate mercury and cadmium (respectively) best. The mercury factor for the crab correlates very closely with that presented by Swanson (1973) for various selected tissues of the purple shore crab (Hemigrapsus nudus) also collected at Dillon Beach. However, the factors for lead (4000) and especially for cadmium (100,000) presented by Brooks and Rumsby (1965) for the blue mussel (Mytilus edulis asteanus) vary from those given here for Mytilus californianus. Perhaps this difference can be accounted on the basis that these authors used "estimated" literature seawater metal levels. Pringle, et al (1968) also report factors for cadmium for Mytilus edulis on a wet-tissue basis and utilizing the same "estimated" seawater values. Assuming that about a six-fold increase in dry versus wet tissue cadmium levels is found for Mytilus californianus and that this factor can be applied to Mytilus edulis in Pringle's study, then one arrives at a factor of about 4800 for cadmium in Mytilus edulis -- a value far more in agreement with this study than with Brooks and Rumsby (1965).

Food Consumption and Tissue Metal Levels in the Sea Star:

Early in the course of the monitoring study, an interesting observation was made in that the tissue-mercury levels in "actively feeding" sea stars (Pisaster ochraceus) were found to be considerably higher than those in "resting" specimens (Table XXIV). It must be noted here that only those specimens which were deemed to be "resting" and collected three to six feet above the splash level have been included in the previously described monitoring study (Tables IV through XIV).

Swanson (1973) has determined that the purple shore crab (Hemigrapsus nudus), when fed the chopped tissues of the mussel (Mytilus californianus) containing natural levels of mercury, was found to contain a rather wide range of levels for the stomach tissue. Although the mean levels of the stomach of the fed animals (0.22 ppm) exceeded those in the starved ones (0.10 ppm), the values were not significantly different by comparison of 95% confidence limits. The same statistical results were found for the gills, hepatopancreas and carapace. The shore crab was then fed the tissues of mussels that had been exposed to seawater solutions 0.1 ppm in mercuric chloride for 96 hours. The whole mussel tissues were determined to contain 3.51 ppm on a wet basis. On consumption of this tissue by the shore crab, analyzed stomach tissue contained about 3.4 ppm mercury --- in essence, a concentration factor less than unity. Thus the consumption of food by the crab cannot account for natural concentration factors normally found for the various tissues of this crab (390 to 1796 times).

In the context of this study an "actively feeding" sea star has been defined as one that has everted its cardiac stomach into or around the prey, usually Mytilus californianus or Pollicipes polymerus. Sea stars with merely the remains of shells or other prey parts held near the mouth by the podia (tube feet) have not been considered to be "actively" consuming since the time elapsed between the sea star's capture and consumption of its prey and the collection of the sea star could not be determined. Also, during that interval the sea star's position might have brought it in contact with wave action while feeding

and thus allowed metal exchange toward the steady state seawater-tissue-metal level by means other than the anal excretory route. In these terms then, a "resting" individual displays neither everted stomach nor prey parts about the mouth.

In gathering specimens for this segment of the study, each active and resting sea star was collected during low tide shortly after sunrise, from at least three to six feet above the water level. Menge (1972) discusses the effects of tide and daylight, as well as season, on the feeding activity of sea stars. In addition, some resting specimens were collected at a depth of about one foot beneath the water surface. No immersed "actively feeding" specimens were taken.

The initial data indicated that perhaps the consumed metal could not to any great extent be excreted by the sea star until it was then reimmersed by the incoming tide. Such data would also lend credence to the fact that the sea star does feed actively at times other than those of immersion and darkness. Once back in the underwater environment, the animal would then re-establish a steady-state equilibrium with the metallic content of the seawater by all its methods of excretion - in essence reaching tissue-metal levels comparable to those in the resting specimens.

With this hypothesis in mind, two large sets of specimens were collected; the results of which are given in Table XXV. Since the sensitivity for lead analysis is less than that for mercury and cadmium and since cadmium and lead trends (Table XXIV) appeared to be roughly the same for feeding versus resting specimens, lead analysis was not performed for these sets.

Unfortunately, the differences in mercury levels were not nearly as pronounced as those from earlier sets, and in some cases actually were reversed. Meanwhile, to further confuse the issue, the cadmium levels of feeding specimens apparently were lower than those in resting ones. Clearly no conclusions (let alone reasons) can be drawn from these combined trends. With respect to all three metals, it is apparent that tissues other than the hepatic caecum must be analyzed. Perhaps some evaluation or correlation could be made if the metal levels of the prey and of the sea star's stomach (cardiac and pyloric) hepatic caecum, and intestine were examined at all stages of ingestion.

Although the feeding and resting individuals surely cannot be statistically separated, it is of interest to note that for mercury, the tissue levels in selected "feeding" individuals is considerably higher than in those resting. In fact no "resting" animal exhibited wet tissue levels above 0.13 ppm of mercury (a range of 0.03-0.13 ppm) with a median value of about 0.10 ppm. Such values correlate very closely with the monitoring values and mean (0.06 ppm) for Dillon Beach specimens (Table XVII). Of the two sets given in Table XXV, the ranges are 0.10 to 0.13 ppm and 0.08 to 0.11 ppm for the resting animals whether collected above or below the water. Thus there appears to be no difference in this latter respect. With regard to the feeding animals it appears that whether the prey is the mussel, Mytilus californianus, or the goose barnacle, Pollicipes polymerus, the mean tissue mercury levels are about the same (Table XXV).

As for tissue cadmium levels in the feeding animals, some values

Table XXIV

Initial Data on "Feeding" and "Resting" Sea Stars

Site & Date	Specimen	Conditions	Mercury (ppm)		Lead (ppm)		Cadmium (ppm)	
			Dry Wt.	Wet Wt.	Dry Wt.	Wet Wt.	Dry Wt.	Wet Wt.
Dillon Beach 6-30-72	1	Feeding(mussel); above water	XXX	0.19	---	---	15.2	XXX
	2	Feeding(mussel); above water	XXX	0.26	2.0	XXX	8.7	XXX
	3	Resting; above water	XXX	0.03	4.4	XXX	9.4	XXX
Land's End 4-8-73	4	Feeding(mussel); above water	0.68	0.12	11.9	2.6	1.78	0.39
	5	Feeding(goose barnacle); above water	0.60	0.12	11.5	2.4	0.96	0.20
	6	Resting; above water	0.10	0.03	11.9	2.7	2.4	0.56
Dillon Beach 4-19-73	7	Feeding(mussel); above water	0.26	0.04	19.6	3.3	1.4	0.24
	8	Resting; above water	0.48	0.08	10.3	1.8	---	---

XXX = not performed
--- = undetectable

Table XXV

"Feeding" and "Resting" Sea Star Study

Site & Date	Specimen	Conditions	Mercury (ppm)		Cadmium (ppm)	
			Dry Wt.	Wet Wt.	Dry Wt.	Wet Wt.
Dillon Beach	9	Feeding(mussel, 1/2 in) above water	1.41	0.29	2.52	0.53
5-19-73	10	Feeding(mussel, 1/2 in) above water	XXX	0.15	XXX	XXX
	11	Feeding(mussel, 1/2 in) above water	0.50	0.17	0.87	0.31
		MEAN	0.95	0.20	1.69	0.42
	12	Feeding(goose barnacle) above water	0.58	0.14	7.0	3.8
	13	Feeding(goose barnacle) above water	0.56	0.09	0.65	0.1
	14	Feeding(goose barnacle) above water	1.57	0.32	1.46	0.30
		MEAN	0.90	0.18	3.03	1.40
	15	Resting; above water	0.77	0.13	—	—
	16	Resting; above water	XXX	0.10	XXX	XXX
	17	Resting; below water	0.58	0.11	2.6	0.52
	18	Resting; below water	0.49	0.10	—	—
		MEAN	0.61	0.11	XXX	XXX

XXX = not performed — = undetectable

Table XXV (continued)

Site & Date	Specimen	Conditions	Mercury (ppm)		Cadmium (ppm)	
			Dry Wt.	Wet Wt.	Dry Wt.	Wet Wt.
Dillon Beach	19	Feeding(mussel); above water	0.67	0.13	0.36	0.07
6-26-73	20	Feeding(mussel,1/2 in) above water	0.46	0.10	2.3	0.53
	21	Feeding(mussel,1/2 in) above water	0.45	0.10	—	—
	22	Feeding(mussel,1/2 in) above water	0.17	0.04	4.3	1.15
	23	Feeding(mussel,3/4 in) above water	XXX	—	XXX	XXX
	24	Feeding(mussel,1/2 in) above water	0.05	0.10	—	—
		MEAN	0.45	0.09	2.32	0.58
	25	Feeding(goose barnacle)above water	0.38	0.07	0.4	0.08
	26	Feeding(goose barnacle) above water	0.13	0.02	—	—
		MEAN	0.25	0.045	0.40	0.08
	27	Feeding[crab(unidentified)] above water	0.60	0.12	0.97	0.17
	28	Resting; above water	0.31	0.09	1.43	0.41
	29	Resting; above water	0.19	0.08	1.01	0.43
	30	Resting; below water	0.39	0.08	6.6	1.3
	31	Resting; below water	0.50	0.11	2.8	0.63
		MEAN	0.34	0.09	3.0	0.69

are higher but most are lower than those in the resting individuals. With respect to the two types of prey, any tissue-metal "trend" is reversed from one set to the other. The very limited data for resting individuals seems to point out that those below water actually contain higher levels than those above it - a result that if proven true could be just as mysterious as the mean tissue-metal differences between feeding and resting specimens. Apriori one should expect that a sea star should be more capable of excretion (by all routes) when immersed rather than when high and dry.

As a summary of the inconclusive data for the feeding sea star, it would seem that with respect to all three metals, such differences are not greater than the individual variation encountered for the resting specimens here or those recorded in Tables IV through XIV. Thus tissue-metal levels are probably not influenced to a great extent by the consumption of prey. Such a result is consistant with that reported by Swanson (1973) for the consumption of the mussel by the purple shore crab.

Introduction to the Accumulation Study:

In the second phase of this project, an attempt was made to determine the source of the three metals for the inhabitants of the mussel bed - whether by adsorption-absorption or consumption or both. In the previous discussion, it was seen that consumption probably does not greatly alter tissue metal levels for the sea star outside the limits of individual variation for a resting specimen. It is quite likely that this is also true for the other invertebrates of the mussel bed. To this end, the mussel (Mytilus californianus), the rock snail (Thais emarginata), and the sea star (Pisaster ochraceus) were selected for an "accumulation" study. These animals were chosen both for convenience in collection and because each represents one level of the consumer order within the mussel bed. "Accumulation" in this context is defined as the combined adsorption and absorption of a metal in an animal since neither process could be separated from the other by the analytical techniques employed here and since tissue metal levels probably reflect both precesses.

Literature:

Discussion of the factors involved in the mobilization and in the tissue accumulation of mercury and other heavy metals has been presented by Brooks and Rumsby (1965), Pringle et al (1968), Clarkson (1972), and others. In general, it is felt that one of the most important routes is that of direct absorption.

Examples of studies considering the accumulation and toxicity of a number of heavy metal ions including those of interest here are

those of Calabrese et al (1973) for the american oyster (Crassostrea virginica), Brown and Ahsanullah (1971) for the brine shrimps (Artemia salina) and the worm (Ophryotrocha labronica), Pringle et al (1968) for a number of marine invertebrates, Wisely and Blick (1967) for a number of marine invertebrate larvae, and Beisinger and Christensen (1972) for the fresh water flea (Dophnia magna). In this latter publication, the authors have been able to correlate decreasing concentrations necessary for reproductive impairment in the water flea with (a) increasing solubility product for the metal sulfide which suggests in vivo chelation of protein mercapto groups, (b) increasing electronegativity of the metal ion, and (c) increasing equilibrium or stability constant of the metal-ATP complex. None of about a dozen other physicochemical constants or parameters could be correlated with such activity.

With particular regard to cadmium, Eisler (1971) has studied its accumulation and actue toxicity in a number of inbertebrates and teleosts common in brackish waters along the eastern U.S. coast. The most resistent to cadmium toxicity, the mummichog or common Killfish (Fundulus heteroclitus), was shown to be more susceptible to cadmium exposures at 20°C than at 5°C and at 5% salinity than 15, 25, or 35%. Dead mummichogs were shown to accumulate 53 and 89 times greater tissue levels of cadmium than live specimens on 24 and 48 hour exposures respectively. In a later publication, Eisler, et al (1972) again reported the cadmium uptake in the tissues of the mummichog and in three invertebrates consumed directly by man. He

concluded that potentially toxic levels of cadmium are accumulated in the adductor muscle of the scallop (Aguipectin irradians), the tail muscles of the lobster (Homarus americanus), and the whole oyster (Crassostrea virginica) on immersion in water containing cadmium concentrations ($<$ 10 ppb) not previously considered hazardous to aquatic life or to public health. Pickering and Gast (1972) have shown the minimum acceptable toxicant concentration for chronic cadmium exposure to the embryos, fry and adults of the fathead minnow (Pimephales promelas) to be 37 ppb.

Gillespie and Scott (1971) and Gillespie (1972) have demonstrated significant but slow mobilization of metallic mercury, mercuric chloride and mercuric sulfide in sediment to the guppy (Puccilia reticulater) under both aerobic and anaerobic conditions. The tissue levels of inorganic and methyl mercury were shown to be consistent with those in fish taken from selected Canadian river systems. Utilizing the oyster (Crassostrea virginica), Cunningham and Tripp (1973) have studied tissue accumulation on forty-five day exposure to 0.01 to 0.10 ppm solutions of mercuric acetate. Whole tissue homogenates were determined to contain 28.0 and 140.0 ppm mercury respectively. Vernberg and Vernberg (1972), having exposed the fiddler crab (Uca pugilator) for twenty-four hours to 0.18 ppm mercury, in seawater, determined gill tissue levels to increase to 1.73 ppm. In a subsequent publication, Vernberg and O'Hara (1973) determined that 82% of the total body burden of mercury was located in the gills.

Swanson (1973) has extensively studied mercury accumulation in the purple shore crab (Hemigrapsus nudus) collected in the Dillon

Beach area. This author's studies centered about the acute toxicity, histopathological changes of various tissues, and patterns of accumulation in selected tissues with respect to salinity and dose of mercuric chloride. Only on long term exposure did the gill lamellae, followed by the antennal glands, show tissue damage. As discussed previously, the various tissues of specimens fed Mytilus californianus containing natural mercury levels could not be statistically separated from those that were starved. In 100% and 25% seawater adjusted to mercury levels of 0.001, 0.01, 0.1, and 0.5 ppm, gill tissues were demonstrated to accumulate incredible amounts, follwoed by the hepatopancreas, stomach and carapace. Dead crabs accumulated very little mercury compared to live specimens. For example, at 24 hours exposure to 0.5 ppm solutions, a comparison of concentration factors for the live versus dead animals shows a ratio of about 1.0 for the carapace, about 35 for the gills and about 5 for the hepatopancreas and stomach. Only very low amounts of mercury were accumulated by live animals placed over dosed sediments and with the exception of gill tissues, concentrations cannot be distinguished from control levels. Finally, the author states that in seawater dosed to 0.001 ppm with mercuric ion, "a concentration considered safe by current public health standards, all tissues examined accumulate mercury above the maximum limit allowable for food products in the United States".

Experimental Procedure:

With regard to experimental procedure in this study, there were

some unavoidable environmental variations for the live animals in the laboratory versus those at the sea shore. The temperature of the water at Dillon Beach usually ranges between 10 and 15°C. This study was carried out in a cold room at 5-6°C. As previously indicated, Eisler (1971) has reported the interesting result that mummichogs (Fundulus heteroclitus) were more susceptable (by factors that ranged between 1.7 to 3.0) to cadmium toxicity at 20°C than at 5°C. However, in this study, it is probable that the low temperature enhanced the toxicity and resulted in greater tissue uptake of metals. At normal temperatures it has been shown that the purple shore crab (Hemigrapsus nudus), and mussel (Mytilus californianus), when exposed to dosed seawater solutions, accumulate far greater mercury levels than found in natural specimens. Also, the short term exposure to such doses is apparently not lethal over that period (Swanson, 1973).

Secondly, each species of animals was immersed in ten gallons of Instant Ocean solution contained in a 13 gallon capacity polyethylene container. In contrast to this, the animals at the seashore are exposed to an extremely large volumn of seawater -- essentially an infinite reservoir of metals at a steady-state concentration. With the low concentrations of the metal used here (1 to 15 times the seawater level — 1-15 X SWL), their absorption by the internal surface of the tank does result in overall lower availability to the animals. Also, as the animals respire and accumulate metal, the overall concentration decreases, and during subsequent hours, a smaller amount is available to each individual animal. The following table (Table XXVI) indicates the loss of mercury and cadmium on the sides of the Nalgene

Table XXVI

Loss of Metals to Tank Walls

Instant Ocean Treatment	Hrs. at which affected	Vol. Instant Ocean Analyzed (ml)	ppm 1 x SWL	ppm 10 x SWL
Control	—	100	—	XXX
Mercury treated	0	100	0.0005	0.0050
	24	100	XXX	0.0030
	48	100	XXX	0.0020
	72	100	0.0002	0.0005
Control	—	100	—	—
Cadmium treated	0	100	0.0015	0.021
	24	100	0.0004	0.018
	48	200	0.0004	0.018
	72	200	0.0004	0.014

tanks containing dosed Instant Ocean only. On account of its low sensitivity to a.a. analysis, the same study for lead was not attempted. However, it is reasonable to assume that the same trends would be found for lead.

Because of the appreciable loss of metal to the tank walls, accumulation studies were limited to periods of only 24 hours.

In order to minimize the difficulties of using dilute metal solutions (hence relatively small total amounts), specimens were chosen that were not large in size (Table II) and the number of animals per tank was limited. Thus by limiting the exposure time and by keeping the volume of seawater much larger than that of the total of animals, it was intended that accumulation would be achieved from a reasonable approximation to the infinite steady-state concentration of the oceanic pool.

After the animals were partially purged of their natural metal content by keeping them in separate, aerated, metal-free Instant Ocean solutions, specimens of each type were immersed in separate tanks containing an Instant Ocean medium of the metal under investigation. These solutions were adjusted to 1X the mean seawater concentration or some multiple of that (usually 5X or 10X) by addition of the appropriate amount of metal. At the appropriate time interval the following number(s) of specimens of a species were removed from the dosed tank: mussels, 2; snails, 5; and sea star, 1 (2 if smaller sizes were used). Each curve (Figures 5-13) was determined only once.

Figure 5

Mytilus californianus: Mercury

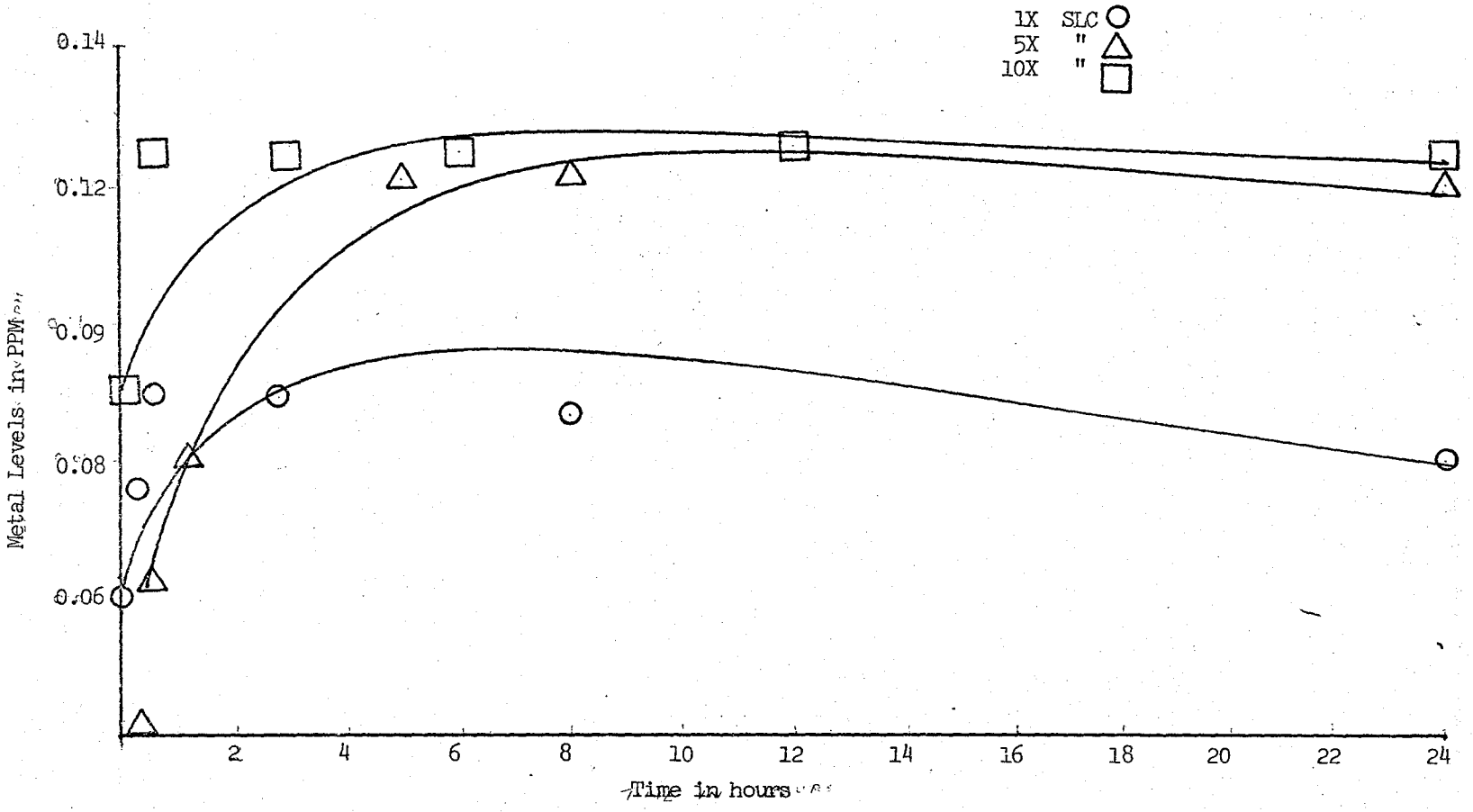
1X SLC
5X "
10X "
0.14
0.12
0.09
0.08
0.06
Metal Levels in PPM
2
4
6
8
10
12
14
16
18
20
22
24
Time in hours

Table XXVII

Accumulation of Mytilus californianus with Regards to Mercury

Name of Species: Mytilus californianus

Metal Accumulated: Mercury

Natural Sea Water Level (SWL): 0.0005 PPM

Time Hrs.	1 X SWL ppm	5 X SWL ppm	7 X SWL ppm	10 X SWL ppm	15 X SWL ppm
0	0.06	0.23	XXX	0.026	0.026
0.25	0.075	0.038	XXX	0.096	XXX
0.5	0.091	0.064	XXX	XXX	XXX
0.75	XXX	XXX	XXX	0.127	XXX
1	0.027	0.080	XXX	---	XXX
3	0.090	---	XXX	0.125	XXX
5	---	0.124	XXX	XXX	XXX
6	XXX	XXX	XXX	0.127	XXX
8	0.085	0.125	XXX	XXX	XXX
12	XXX	XXX	XXX	0.129	XXX
24	0.078	0.122	XXX	0.123	0.181
		Instant Ocean			
0	0.0005	0.0028	XXX	0.0048	0.008
24	0.0002	0.0025	XXX	0.0027	0.0068

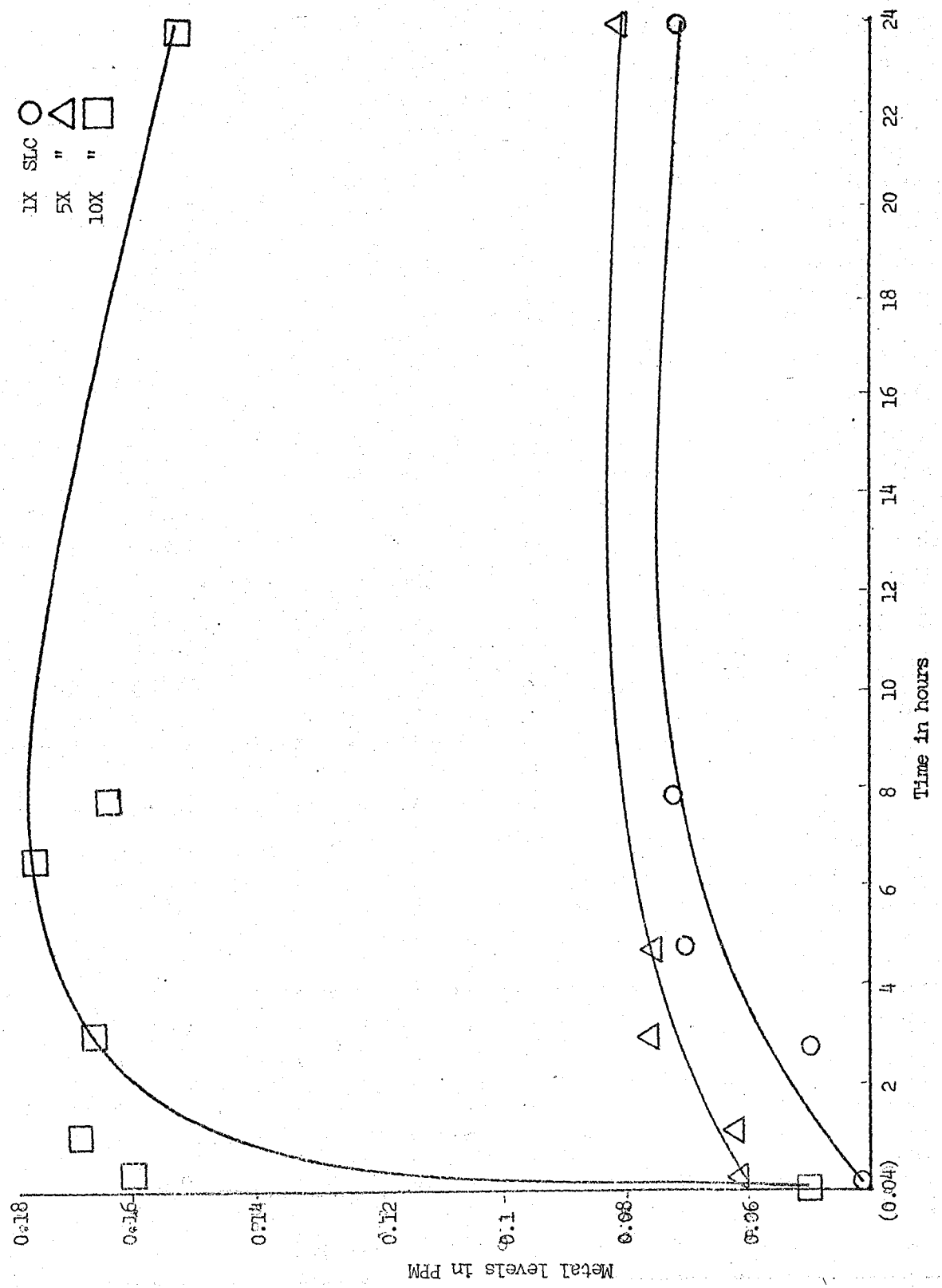
1X SLC
5X "
10X "
Metal levels in PPM
0.18
0.16
0.14
0.12
0.1
0.08
0.06
(0.04)
2
4
6
8
10
12
14
16
18
20
22
24
Time in hours

Table XXVIII

Accumulation of Thais emarginata with Regards to Mercury

Name of Species: Thais emarginata

Metal Accumulated: Mercury

Natural Sea Water Level (SWL): 0.0005 PPM

Time Hrs.	1 X SWL PPM	5 X SWL PPM	7 X SWL PPM	10 X SWL PPM	15 X SWL PPM
0	---	---	0.10	0.052	0.052
0.83	XXX	XXX	0.116	0.162	XXX
0.25	XXX	0.063	XXX	0.162	XXX
1.0	0.031	0.059	0.125	0.170	XXX
3.0	0.053	0.078	0.114	0.171	XXX
5.0	0.072	0.078	0.112	XXX	XXX
6	XXX	XXX	XXX	0.181	XXX
8	0.077	---	0.113	0.168	
24	0.075	0.081	0.112	0.151	0.308

Instant Ocean

0	0.0005	0.0028	0.0034	0.0048	0.008
24	0.0003	0.0024	0.0025	0.0037	0.0068

Figure 7

Pisaster ochreaceus: Mercury

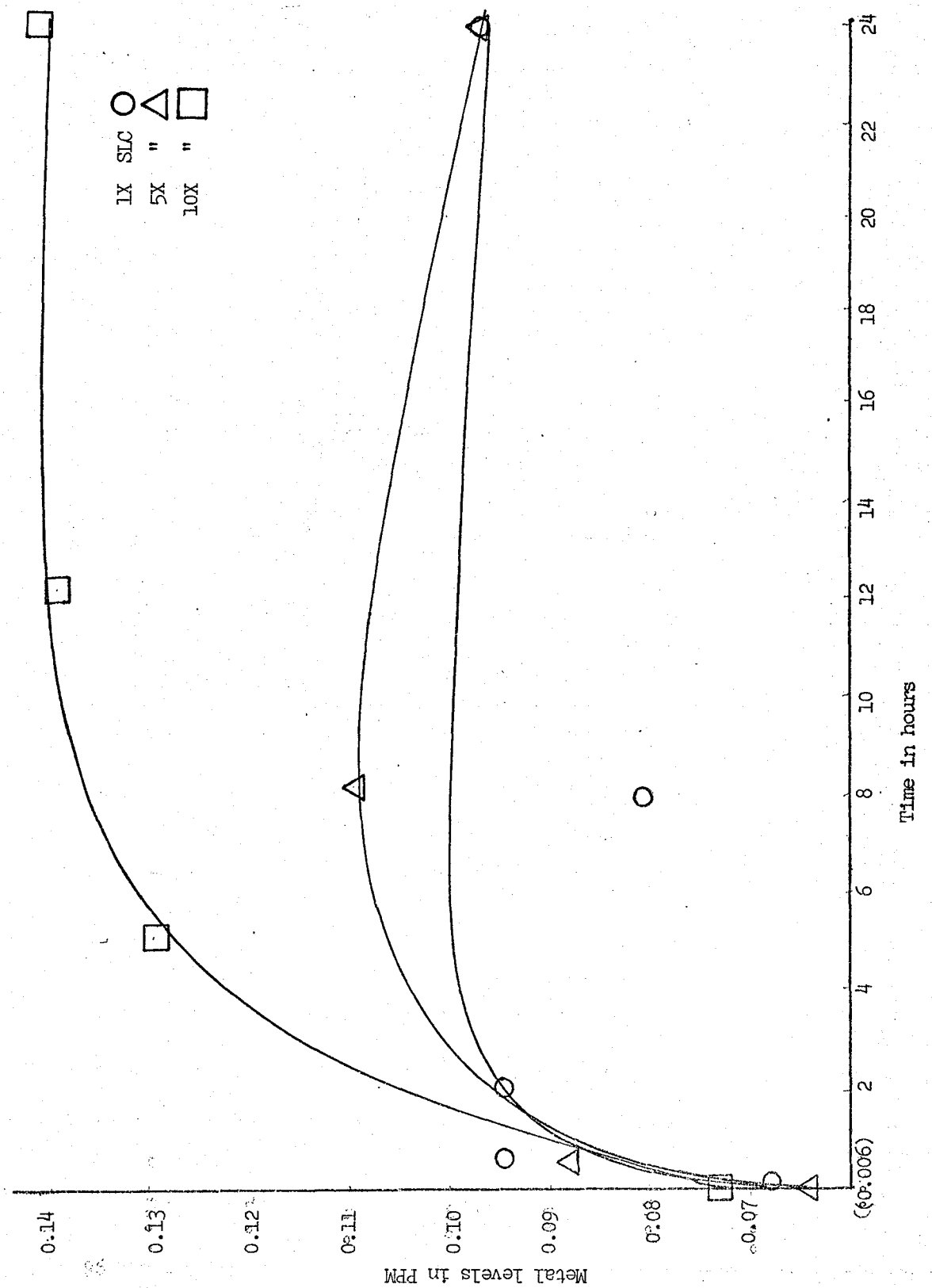
1X SLC
5X "
10X "
Metal levels in PPM
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
(0.006)
2
4
6
8
10
12
14
16
18
20
22
24
Time in hours

Table XXIX

Accumulation of Pisaster ochreaceus with Regards to Mercury

Name of Species: Pisaster ochreaceus

Metal Accumulated: Mercury

Natural Sea Water Level (SWL): 0.0005 PPM

Time Hrs.	1 X SWL PPM	5 X SWL PPM	7 X SWL PPM	10 X SWL PPM	15 X SWL PPM
0	0.068	0.064	XXX	0.073	0.073
0.5	0.094	XXX	XXX	XXX	XXX
0.75	XXX	0.087	XXX	XXX	XXX
2	0.094	—	XXX	XXX	XXX
5	XXX	XXX	XXX	0.129	XXX
8	0.083	0.113	XXX	XXX	XXX
12	XXX	XXX	XXX	0.139	XXX
24	0.101	0.101	XXX	0.144	0.180
		Instant Ocean			
0	0.0005	0.0026	XXX	0.0078	0.008
24	0.0002	0.0025	XXX	0.0037	0.0065

Blank spaces represent analyses not performed

(—) represent non-detectable levels

Figure 8

Mytilus californianus: Cadmium

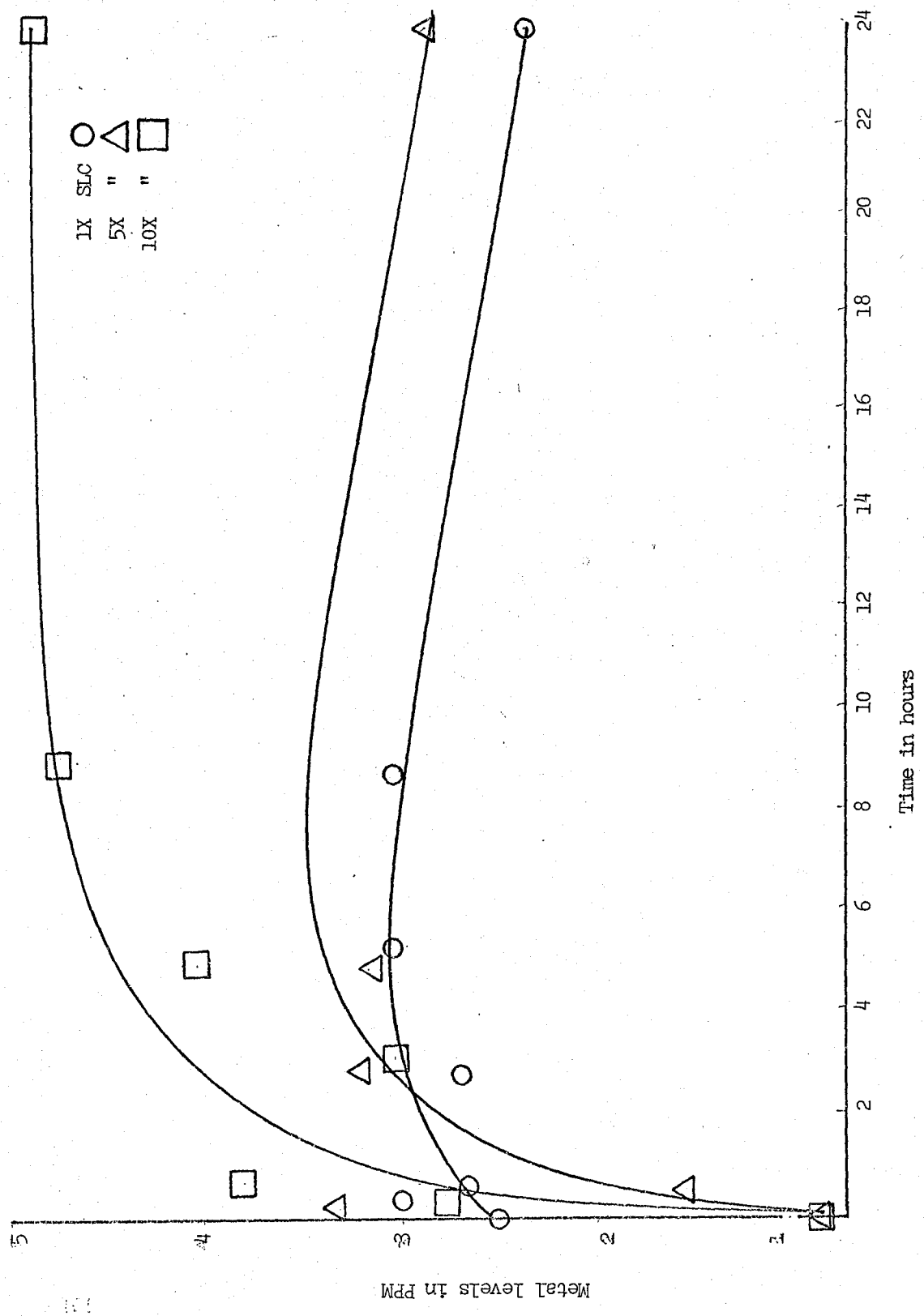
1X SLC
5X " "
10X " "
Metal levels in PPM
Time in hours
2
4
6
8
10
12
14
16
18
20
22
24

Table XXX

Accumulation of Mytilus californianus with Regards to Cadmium

Name of Species: Mytilus californianus

Metal Accumulated: Cadmium

Natural Sea Water Level: 0.0016 PPM

Time Hrs.	1 X SWL PPM	5 X SWL PPM	7 X SWL PPM	10 X SWL PPM	15 X SWL PPM
0	2.48	0.01	2.90	0.61	0.61
0.25	3.08	3.33	3.05	2.77	XXX
0.50	2.59	1.62	XXX	3.86	XXX
3	2.62	3.26	3.14	3.02	XXX
5	3.20	3.16	3.73	4.01	XXX
9	3.16	XXX	XXX	4.68	XXX
24	2.41	2.98	3.77	4.89	3.47

Instant Ocean

0	0.0015	0.0085	0.012	0.0180	0.025
24	0.0011	0.0065	0.010	0.0120	XXX

Figure 9

Thais emarginata: Cadmium

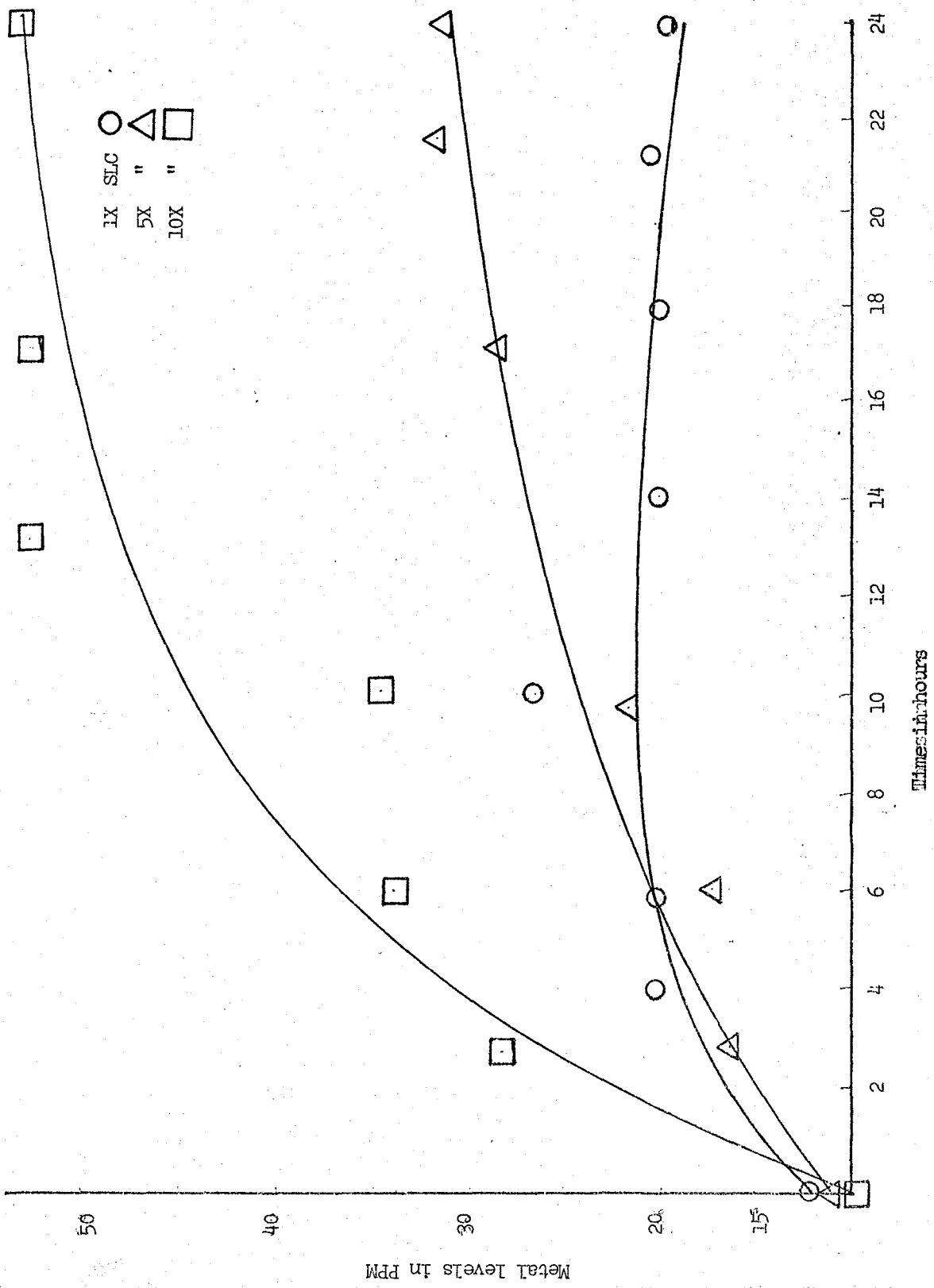
1X SLC
5X "
10X "
50
40
30
20
15
Metal levels in PPM
2
4
6
8
10
12
14
16
18
20
22
24
Time in hours

Table XXXI

Accumulation of Thais emarginata with Regards to Cadmium

Name of Species: Thais emarginata

Metal Accumulated: Cadmium

Natural Sea Water Level (SWL): 0.0016 PPM

Time Hrs.	1 X SWL PPM	5 X SWL PPM	7 X SWL PPM	10 X SWL PPM	15 X SWL PPM
0	12.3	10.6	XXX	9.97	9.97
3.0	20.6	16.9	XXX	27.9	XXX
6.0	20.0	18.2	XXX	33.8	XXX
10.0	26.8	22.0	XXX	35.1	XXX
13.0	XXX	62.5	XXX	53.2	XXX
14.0	20.1	XXX	XXX	XXX	XXX
17.0	XXX	28.8	XXX	53.4	XXX
18.0	20.5	XXX	XXX	XXX	XXX
21.0	21.1	21.9	XXX	11.0	XXX
24.0	20.0	29.5	XXX	53.5	120.8 *
			Instant Ocean		
0	0.0016	0.0075	XXX	0.017	0.025
24	0.0009	0.0058	XXX	0.011	0.025

* appeared to be dead

Figure 10

Pisaster ochreaceus: Cadmium

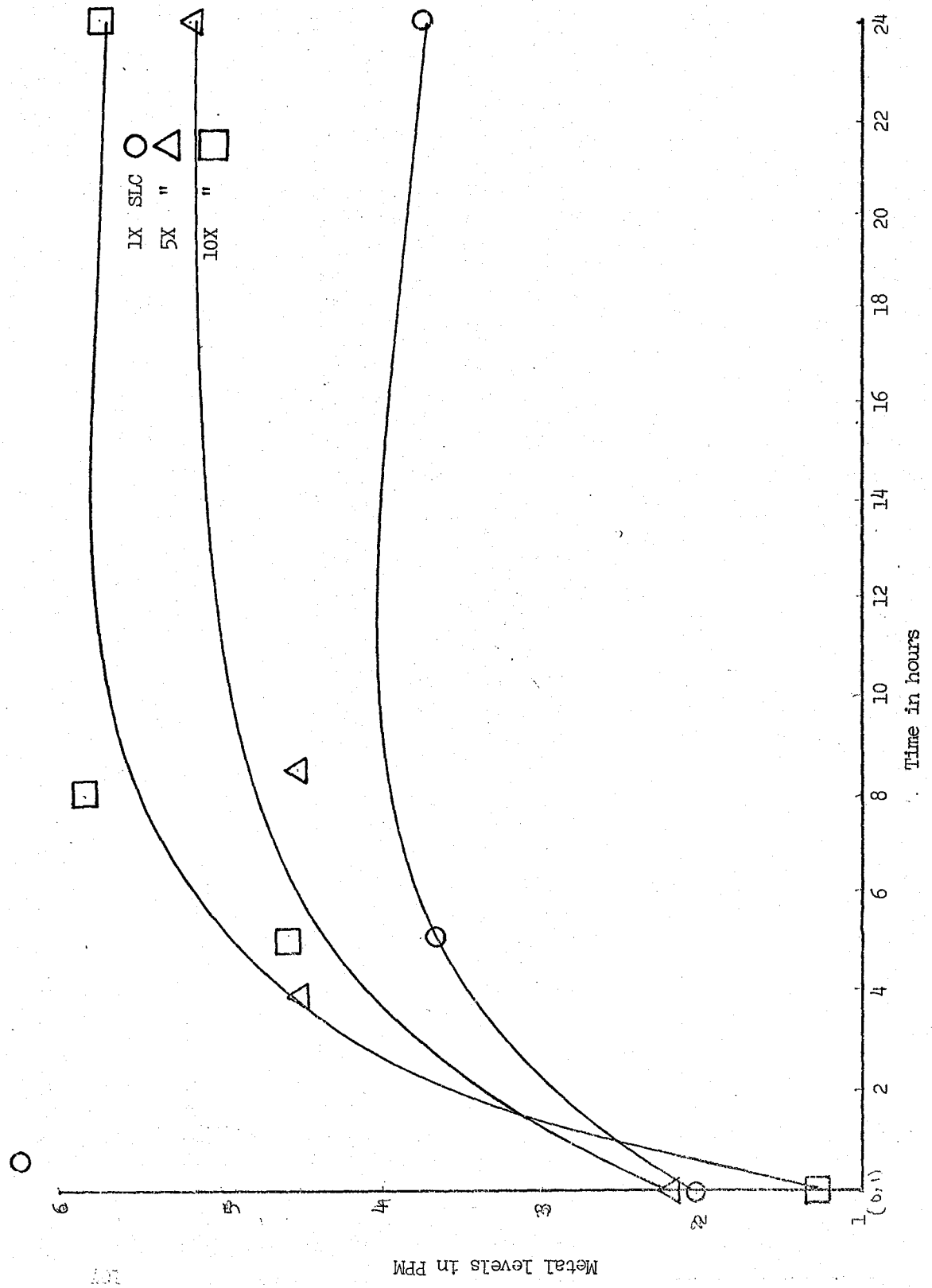
1X SLC
5X "
10X "
Metal levels in PPM
Time in hours
(0,1)
2
3
4
5
6
2
4
6
8
10
12
14
16
18
20
22
24

Table XXXII

Accumulation of Pisaster ochreaceus with Regards to Cadmium

Name of Species: Pisaster ochreaceus

Metal Accumulated: Cadmium

Natural Sea Water Level (SWL): 0.0016 PPM

Time Hrs.	1 X SWL PPM	5 X SWL PPM	7 X SWL PPM	10 X SWL PPM	15 X SWL PPM
0	2.08	XXX	2.34	1.32	1.32
	7.33	XXX	XXX	XXX	XXX
3.5	XXX	XXX	4.52	XXX	XXX
5.0	3.77	XXX	XXX	4.59	XXX
8.0	XXX	XXX	4.53	5.89	XXX
24.0	3.89	XXX	5.28	5.83	4.67

Instant Ocean

0	0.0015	XXX	0.0104	0.015	0.025
24	0.0009	XXX	0.0100	0.012	XXX

Figure 11

Mytilus californianus: Lead

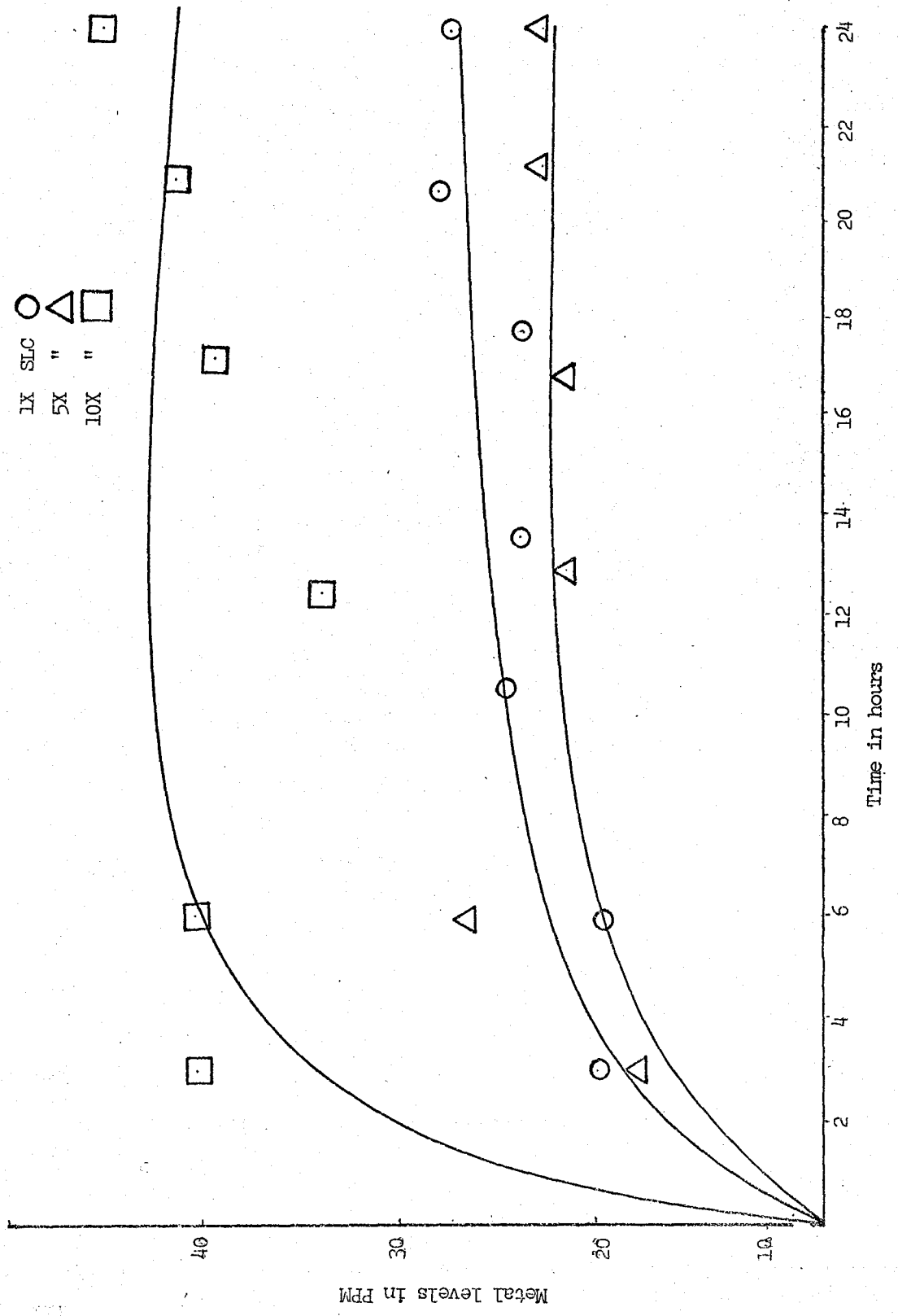
1X SLC
5X "
10X "
Metal levels in PPM
10
20
30
40
Time in hours
2
4
6
8
10
12
14
16
18
20
22
24

Table XXXIII

Accumulation of Mytilus californianus with Regards to Lead

Name of Species: Mytilus californianus

Metal Accumulated: Lead

Natural Sea Water Level(SWL): 0.0016 PPM

Time Hrs.	1 X SWL PPM	5 X SWL PPM	7 X SWL PPM	10 X SWL PPM	15 X SWL PPM
0	---	---	XXX	---	---
3	20	18	XXX	40	XXX
6	19	26	XXX	40	XXX
10	25	XXX	XXX	XXX	XXX
12.5	XXX	XXX	XXX	34	XXX
13	XXX	21	XXX	XXX	XXX
14	24	XXX	XXX	XXX	XXX
17	XXX	21	XXX	39	XXX
18	24	XXX	XXX	XXX	XXX
21	28	23	XXX	43	XXX
24	27	23	XXX	45	46

Instant Ocean

0	XXX	XXX	XXX	0.017	XXX
24	XXX	XXX	XXX	0.002	XXX

Figure 12

Thais emarginata: Lead

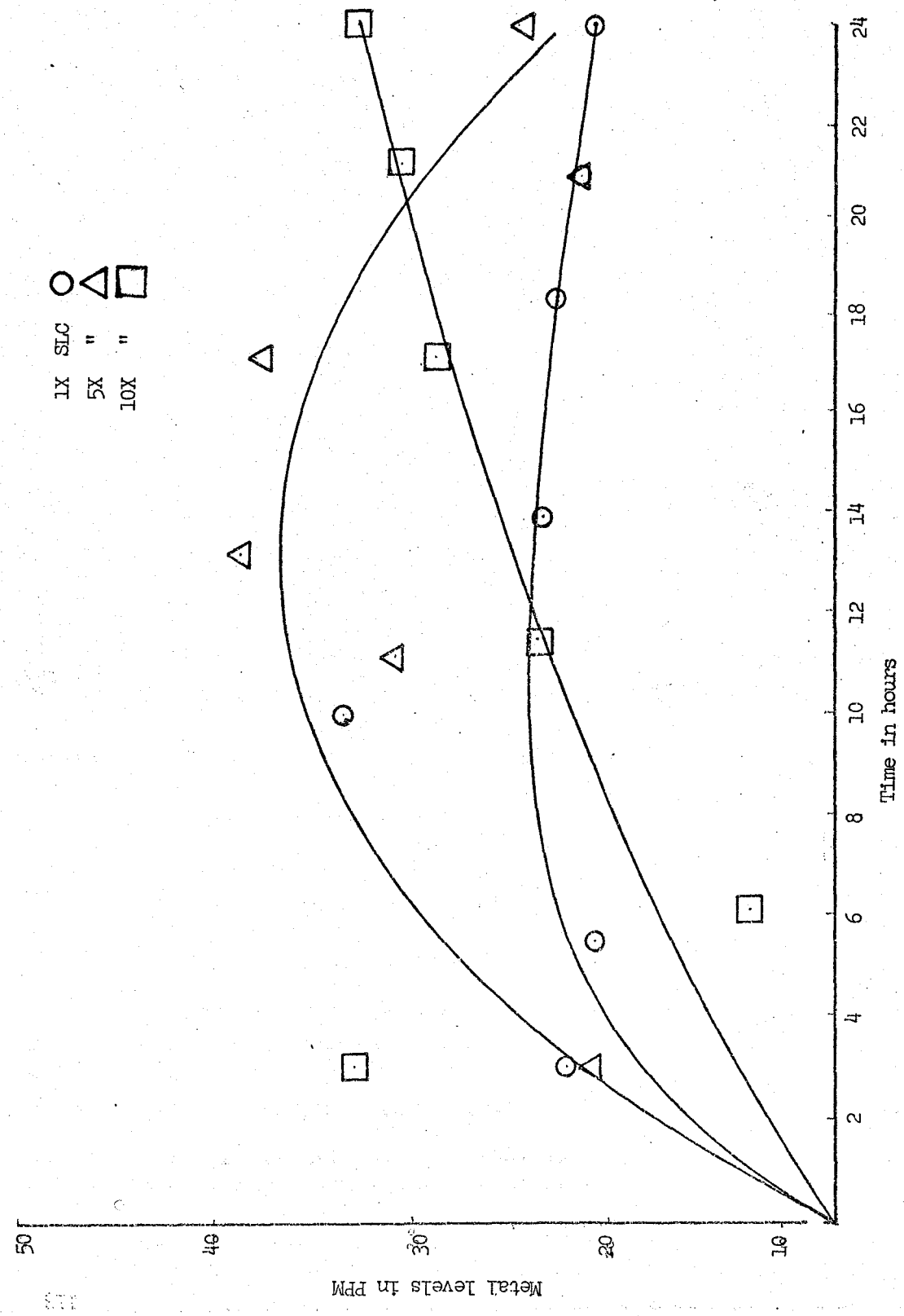
1X SLC
5X "
10X "
Metal levels in PPM
50
40
30
20
10
2
4
6
8
10
12
14
16
18
20
22
24
Time in hours

Table XXXIV

Accumulation of Thais emarginata with Regards to Lead

Name of Species: Thais emarginata

Metal Accumulated: Lead

Natural Sea Water Level (SWL): 0.0016 PPM

Time Hrs.	1 X SWL PPM	5 X SWL PPM	7 X SWL PPM	10 X SWL PPM	15 X SWL PPM
0	---	---	XXX	---	---
3	2.3	2.2	XXX	3.3	XXX
5.5	2.0	XXX	XXX	XXX	XXX
6	XXX	---	XXX	1.4	XXX
10	3.4	XXX	XXX	XXX	XXX
11	XXX	3.0	XXX	2.4	XXX
13	XXX	3.8	XXX	XXX	XXX
14	2.4	XXX	XXX	5.4	XXX
17	XXX	3.7	XXX	2.8	XXX
18	2.3	XXX	XXX	XXX	XXX
21	2.1	2.1	XXX	3.0	XXX
24	2.0	2.5	XXX	3.2	6.0
			Instant Ocean		
0	XXX	XXX	XXX	0.017	0.03
24	XXX	XXX	XXX	0.002	XXX

Figure 13

Pisaster ochreaceus: Lead

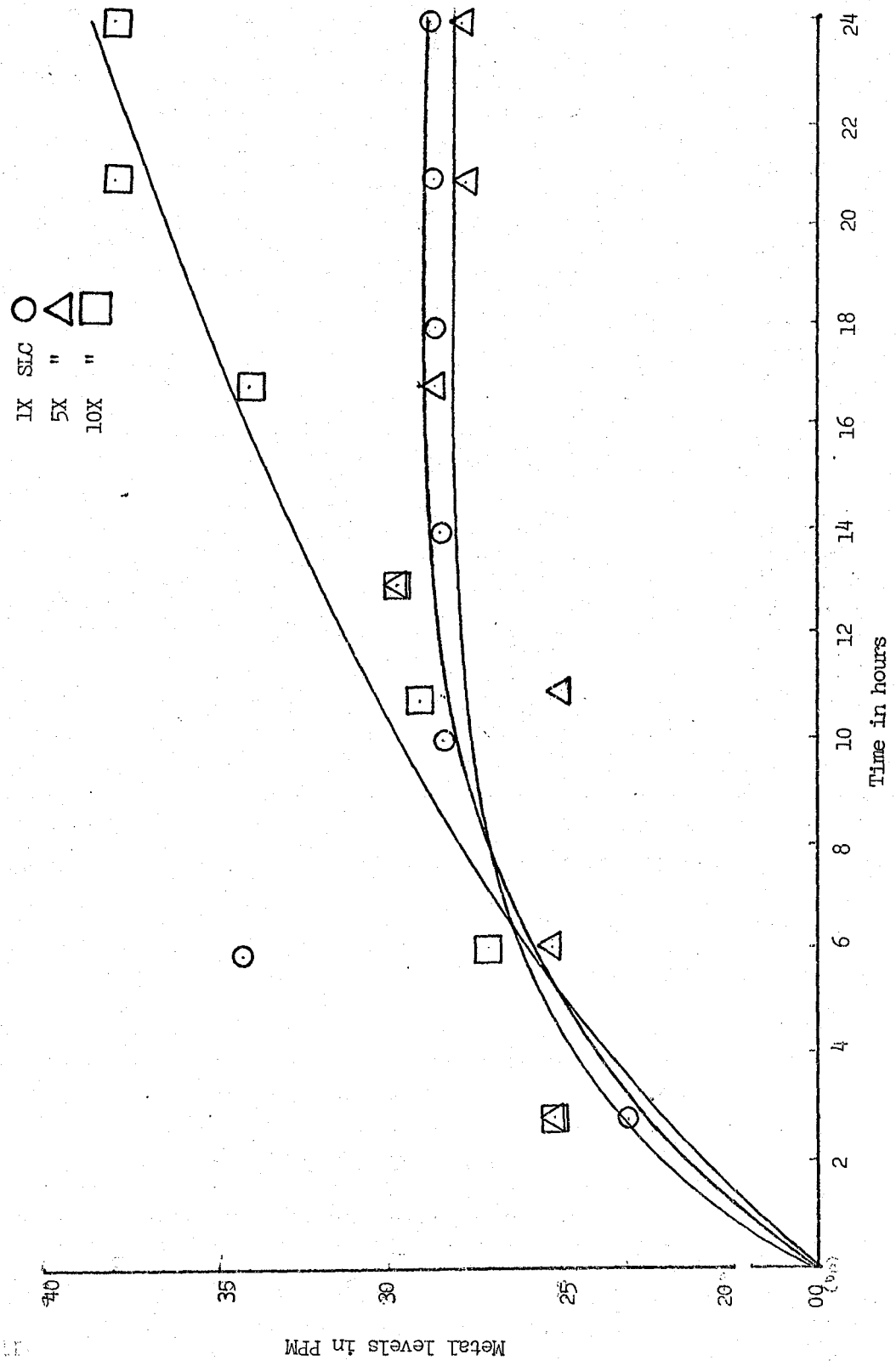
1X SLC
5X "
10X "
Metal levels in PPM
Time in hours
40
35
30
25
20
00
2
4
6
8
10
12
14
16
18
20
22
24

Table XXXV

Accumulation of Pisaster ochreaceus with Regards to Lead

Name of Species: Pisaster ochreaceus

Metal Accumulated: Lead

Natural Sea Water Level (SWL): 0.0016 PPM

Time Hrs.	1 X SWL PPM	5 X SWL PPM	7 X SWL PPM	10 X SWL PPM	15 X SWL [1] PPM
0	---	---	XXX	---	---
3	23	25	XXX	25	XXX
6	34	25	XXX	27	XXX
10	28	XXX	XXX	XXX	XXX
11	XXX	24	XXX	29	XXX
13	XXX	29	XXX	29	XXX
14	28	XXX	XXX	XXX	XXX
17	XXX	28	XXX	34	XXX
18	28	XXX	XXX	XXX	XXX
21	28	27	XXX	38	XXX
24	28	27	XXX	38	46

Instant Ocean

0	XXX	XXX	XXX	0.017	XXX
24	XXX	XXX	XXX	0.002	XXX

Blank spaces represent analyses not performed

(---) represent non detectable levels

1 represents approximately 15 X SWL

Results for Accumulation by Live Animals:

The results of the accumulation of the three metals in the three species of animals are presented in Tables XXVII to XXXV and Figures 5 to 13. Analysis of either the data or curves suggests that in most instances for mercury, each of the animals accumulates to a "steady-state" level by the first hour of exposure, while for cadmium and lead, the same occurs within three hours. This correlation of steady-state tissue levels and the time needed to achieve the same holds well for the lower seawater doses (1X, 5X) but some exceptions are noted for the 10X curves where a greater time is required. Note with particular regard to Figures 5-13 that the steady-state portion of each set of curves shows a consistent trend to relatively smaller amounts of metal accumulated by tissue with larger concentrations of metal in the medium -- in essence, a non linear dose to response (accumulation) relationship. Such an inverse response rules out simple passive accumulation alone and does suggest that some active excretory process is operative.

The mean steady-state tissue levels for the three animals immersed in solutions containing either 1X or 10X the metal present in normal seawater are presented in Table XXXVI along with the enrichment factor for each. The normal seawater levels (SWL) were determined from samples taken from Dillon Beach from November 1972 through April 1973 (Table XXI). The mean steady-state tissue mercury values are calculated by averaging values from hours one through twenty-four inclusive, while the cadmium and lead values are found by considering all values from hours three

through twenty-four inclusive. With particular regard to the 1X accumulation, these tissue levels should correlate with those observed for the wet season monitoring from Dillon Beach (Table XXI), assuming for the moment that the environmental to laboratory temperature differences can be ignored. In this respect, mean accumulation levels for mercury and lead are close to (but somewhat higher than) the mean monitoring levels; however, they generally fall within or are very close to the range of the latter values in Table XXI. Conversely, the mean accumulation level for cadmium (with the exception of Mytilus californianus which correlates very well) is almost twice that for mean monitoring value in Thais emarginata, and is almost one-half that for the mean monitoring value in Pisaster ochreaceus. For both Pisaster ochreaceus and Thais emarginata, the former values fall just within the range of the monitoring values. Comparison of the 10X mean cadmium accumulation level for the sea star with the mean monitoring data gives a closer correlation but the former value still remains below the latter. Similar comparison, of 10X data for all three animals for mercury and lead, and the mussel and snail for cadmium results in an increasingly divergent (if any) relationship -- all accumulation values are considerably higher than and usually outside of the monitoring value ranges.

In summary, there is good correlation between the 1X accumulation (even at temperatures some 5-10°C below normal) and the monitoring studies with the exception of cadmium in two animals. It is probable that the somewhat higher accumulated tissue levels are a

Table XXXVI

The Mean, Range and Concentration Factor for Steady-State Tissue-Metal Levels

	Mercury Wet Wt. Basis (ppm)		Lead Dry Wt. Basis (ppm)		Cadmium Dry Wt. Basis (ppm)	
	1X SWL	10X SWL	1X SWL[a]	10X SWL	1X SWL	10X SWL
Mytilus californianus	0.07 (0.027-0.09) 140	0.13 (0.123-0.129) 27	23.8 (19-28) 14000	40.2 (34-45) 2365	2.84 (2.41-3.16) 1893	4.15 (3.02-4.89) 230
Thais emarginata	0.06 (0.031-0.077) 120	0.17 (0.151-0.181) 35	23.6 (20-34) 13870	30.7 (14-54) 1816	21.3 (20.0-26.8) 13300	38.2 (11.0-53.5) 2245
Pisaster ochreaceus	0.09 (0.083-0.101) 180	0.14 (0.129-0.144) 29	28.1 (23-34) 16520	31.4 (25-38) 1846	3.83 (3.77-3.89) 2553	5.43 (4.59-5.89) 362

a The 1X concentration was not verified by a.a. analysis but is assumed to be 1.7 ppb.

result of the lower experimental temperature and a decreased ability to actively excrete the metal. The non-linear dose-response (tissue uptake) relationship as well as the decrease in accumulation or enrichment factors as the dose is increased tend to lend credence to a tissue accumulation that is not just passive but probably must include some active excretory mechanism. Swanson (1973) has reported this same negative dose-response relationship and decreasing enrichment factors when the purple shore crab is exposed to increasing doses of mercury in 25 and 100% seawater. Apparently the major source of these three metals to tissues of the three animals considered here is by accumulation, whether active or passive. Again, as has been previously demonstrated for the "feeding" sea star, food consumption by either of the three animals can only cause small (if any) and fleetingly transient effects on the tissue metal levels since the controlled accumulation study (where the animals have been starved and purged in metal-free water for 2-3 days before beginning the study) correlates quite closely with the levels observed in the monitoring study. And finally, with regard to the lowered temperature as it affects the 1X accumulation versus the monitoring enrichment factors and apparent toxicity, the monitoring and accumulation factors are in good agreement (see Tables XXIII and XXXVI) -- the only exception being cadmium in the snail. The toxicity appears to be enhanced since for each of the metals at the 15X level, all three animals tended to expire. Swanson (1973) and other authors previously cited, have demonstrated that for many of the same or closely related species at normal environmental temperatures,

that lethal toxic effects were not found at such low doses and short term exposures.

For each of the three metals used in this short term accumulation study it is apparent that the tissue metal levels do not change considerably once a steady-state has been reached. The tissue level drop seen in some cases at the 24-hour mark (Figures 5-13) is probably due to redistribution of the metal from tissue to loss at the tank wall. In retrospect it appears that it would be difficult or even erroneous to attempt to extrapolate from the tissue levels of any of these three animals back to the seawater level of any of the three metals considered.

Results for Accumulation by Dead Animals:

This study was done to determine whether accumulation is an "active" or a passive process and if these two processes can be separated experimentally. Presumably, without an active excretory mechanism, the tissue levels in the dead animals would reflect a passive diffusion to some equilibrium tissue level -- probably to a value higher than that observed as a steady-state level in live specimens.

On the day of the experiment, specimens that had been sacrificed by freezing were thawed and used in the following manner: in the case of the sea star, a single animal was used for each reading at 24 and 48 hours; for the mussels, two animals were used for each reading; and for the snails, at least 4 animals were used for each reading. This study was carried out at approximately (unmeasured) the 10X

Table XXXVII

Relative Accumulation of Mercury, Lead, and Cadmium by Dead Animals

Name of Species	Mercury (ppm)[b]				Lead (ppm)[c]				Cadmium (ppm)[c]			
	0 hr[a]	24 hr.		48 hr.	0 hr[a]	24 hr.		48 hr.	0 hr[a]	24 hr.		48 hr.
	Dead	10X SLC Live[d]	app.10X SLC Dead[e]	app.10X SLC Dead[e]	Dead	10X SLC Live[d]	app.10X SLC Dead[e]	app.10X SLC Dead[e]	Dead	10X SLC Live[d]	app.10X SLC Dead[e]	app.10X SLC Dead[e]
Mytilus californianus	0.09	0.12	0.16	0.11	21.3	45.0	19.5	36.6	6.26	4.89	5.03	3.57
Thais emarginata	0.05	0.15	0.23	0.10	18.9	32.0	27.0	37.9	12.60	53.5	10.2	29.7
Pisaster ochreaceus	0.07	0.14	0.04	0.06	19.7	38.0	23.5	29.2	6.6	5.83	5.06	7.26

a. Average of 3 readings on individual specimen obtained from the monitoring data, Series VII, Dillon Beach (Table X).

b. Results based on wet weight analysis

c. Results based on dry weight analysis

d. The data is reproduced from corresponding accumulation study (Tables XXVII-XXXV)

e. This study was done in instant ocean at approximately 10X SLC

seawater concentration for each metal in order to minimize metal loss to the tank walls over the 48 hour accumulation period. As before, this study was also carried out at 5-6°C. Animals for the "dead" accumulation study were collected with those for monitoring series VII (April 19, 1973).

The results of this study are given in Table XXXVII. On initial inspection, the data is not at all clear-cut. For each dead animal and every metal, it appears that the rate of passive diffusion of the metal into the tissues to achieve equilibrium levels requires far more than the one to three hours previously required to reach steady-state levels in the live animals. With the exception of cadmium and mercury in the mussel and mercury in the snail, tissue levels at 48 hours were still increasing when compared to the 24 hour level. It is also quite possible that for mercury in each of the two above animals that the apparent decrease is really loss to the tank walls. Although in most cases the 48 hour tissue levels are not as great as those for the live animals at 24 hours, the levels are increasing rapidly - especially for lead and cadmium. Unfortunately the experimental design of not refurbishing metal concentrations every 24 hours coupled to the fact that beyond 24 hours there are increasingly large losses to the tank walls does not allow for an absolute answer here. However, if these problems could be circumvented or if extrapolation of the preceeding data is permissible, it might be expected that at 72 or 96 hours an equilibrium would be reached when tissue levels would be indeed greater in the dead animals. There are only two cases

here where passive diffusion appears to be rapid enough to cause higher tissue levels in the dead specimens. These are for the 24 hour exposure of the mussel and the snail to mercury. If the general trend toward increasing "dead" levels on exposures of over 24 hours is coupled with this last observation where "dead" equilibrium levels are definitely higher than "live" steady-state ones, it seems very possible that indeed an "active" excretory mechanism has been abolished when these animals are sacrificed before exposure.

Also, it must not be forgotten that the anotomical state of the animal can play a large role in the exposure of its tissues to dissolved chemicals. With a dead mussel, the shell is open and almost all of its tissues are in intimate contact with the medium. The snail, while its withdrawn foot may be somewhat exposed, generally shields all its other organs within its shell. Finally for the sea star, the hepatic caecum is deep within each very impervious arm. Thus, without normal respiration and circulation in the live animal, distribution to the tissues, let alone passive diffusion into them, becomes increasingly difficult.

It is of interest (but of questionable importance) that mercury apparently was accumulated as a far greater rate in each of the live ($\backsim$1 hr. to steady-state) and in 2 of 3 of the dead ($\backsim$24 hrs. to equilibrium) animals. Comparatively and respectively, cadmium and lead require at least 3 hours to steady-state levels and far greater than 48 hours to equilibrium.

Unfortunately the 10X "dead" accumulation was also determined only once, the water-metal levels were not monitored, and a 1X ex-

posure was not done.

Eisler (1971) has reported 53 and 89 times increased tissue levels of cadmium in the dead mummichog (Fundulus heteroclitus) as compared to the live, control specimen on 24 and 48 hour exposures, respectively, to a 40 ppm cadmium solution at 20°C. In other experiments, the author has varied dose, temperature and salinity and has found that the live killifish "contained progressively smaller percentages of the total cadmium available in the medium with increasing cadmium levels, whereas fish that had died contained progressively larger percentages". As previously stated, these fish proved to be more susceptible to cadmium toxicity at 20°C than at 5°C and at 5% salinity rather than at 15, 25, and 35% for either temperature.

On the other hand, Swanson (1973) has shown for dead specimens of the purple shore crab (Hemigrapsus nudus) exposed to 0.5 ppm mercury seawater solutions of both 25 and 100% salinity over a 36 hour interval, that tissue levels and concentration factors were lower (by approximately 1 to 35 X's) than for live specimens exposed to the same conditions. However, at 36 hours exposure, the tissue levels in both the live and dead specimens were still increasing. What the levels would have been when steady-state or equilibrium had been reached is difficult to say since such a study of dead animals is limited to not more than a few days because of bacterial decomposition of the specimens. Thus, comparisons can be made only for the short term 36 hour exposure — a time period perhaps not great enough to draw definite conclusions with regard to the purple shore crab, or similarly, to the animals considered herein.

SUMMARY

In the first phase of this two-part project, the monitoring of mercury, lead and cadmium was performed in selected members of the "Mytilus, Pollicipes, Pisaster association" collected from the Dillon Beach and Land's End areas of California. The following observations could be made from this portion of the study:

1. Metal levels in animals of the same species collected on the same day, or during the same season were often quite different. Thus, any attempt to establish a base line for these metals must be done in the light of the fact that there exists considerable individual variation within each species even though collected on the same day or during the same season. (Tables IV-XIV).

2. Mercury levels in animals investigated from both the sites ranged from 0.01-0.93 ppm and those of lead and cadmium ranged from 1.0-31.9 ppm and 0.3-23.2 ppm, respectively.

3. Pisaster ochreaceus, the tertiary consumer at the apex of the mussel bed food chain, was not found to contain the highest levels of metals. The porcerlain crab (Petrolisthes cinctipes) was found to contain by far the highest levels of mercury. Mercury levels in the remaining animals were an order of magnitude less than in the porcerlain crab and were in close proximity of each other. In regards to lead and cadmium,

the tissue levels were about the same for all and values for the sea star were not the highest (Table XVII). Thus, in contrast to the earlier assumption, these results led us to believe that pyramiding may not be operative through the trophic levels of the mussel bed consumer chain on the wet and dry tissue weight basis.

4. An attempt was made to compare the mean metal levels of various animals (Table XVII) with their total protein content (Table XVIII) on both a wet and dry weight basis. The porcerlain crab (Petrolisthes cinctipes) had the highest protein level and also the highest mercury level on both a wet and dry weight basis. The purple rock snail (Thais emarginata), a secondary consumer, stood next in its wet protein content, and was consistently high in its metallic levels. The remaining animals have a relatively low "wet" protein content (nearly all in the same range) and also have about the same tissue metal levels.

5. In contrast to our original assumption, the metal levels in the various species and seawater collected from "maximum polluted Land's End" and "minimum polluted Dillon Beach" were found to be quite close to each other on a wet and dry weight basis (Table XVII).

6. On comparing the tissue and sea water levels of the metals on the basis of the Dry (June-October) and Wet (November-April) seasons, it was found that mercury levels in the

different species at two sites remained about the same during both the seasons, but that there were considerable increases in lead and cadmium levels during the "wet" months as compared to "dry" months. (Tables XXI-XXII).

7. On the basis of concentration factors (the ratio of tissue to water levels of a metal), it was observed that almost all the animals under investigation were equally capable of accumulating lead, yet the porcerlain crab and goose barnacle appear to accumulate mercury and cadmium more readily. (Table XXIII).

8. The tissue-mercury levels in "actively feeding" sea stars (Pisaster ochreaceus) were found to be higher but not significantly different from those in "resting" animals. Cadmium was also studied but no meaningful relationship between the metal levels in "actively feeding" and "resting animals" could be seen (Tables XXIII-XXIV). Lead was not considered for this study.

In the second phase of this project, an attempt was made to determine the mechanism of transfer of metals from marine environments to mussels (Mytilus californianus), snail (Thais emarginata), and sea star (Pisaster ochreaceus). This could be absorption, adsorption, or perhaps a combination of both absorption and adsorption. This study led us to the following observations:

9. All three animals reached a steady state within the first hour of exposure to mercury (sea water level or a multiple of that) in the tank containing Instant Ocean while the same steady state for cadmium and lead was reached in about three hours (Figures 5-13, Tables XXVII-XXXV).

10. The lack of a linear dose-response relationship for each metal in every animal as indicated in the curves (Figures 5-13), as well as a decrease in enrichment factors as dose is increased, rule out the possibility that passive accumulation alone is responsible for the tissue levels observed.

11. Finally, in order to determine whether accumulation is an active or passive process, a study was done at 10X sea water level (unmeasured) with dead mussels, snails, and sea stars. With the exception of cadmium and mercury in the mussel and mercury in the snail, higher tissue levels were found in the species exposed for 48 hours as compared to those exposed for only 24 hours. In two cases, species of mussels and snails exposed to mercury for 24 hours contained sufficiently high metal levels to indicate that passive diffusion may be responsible for these higher levels. Thus, these two observations, coupled together, indicated that the "active" excretion mechanism had been abolished in these dead animals.

The results of this two-part project have contributed some basic knowledge about the metallic levels in the non-mobile inhabitants of

coastal mussel beds at the sites investigated in this study. To some degree, this study also has documented some of the mechanisms by which these ecologically significant animals acquire metals. Hopefully, this research will help to define and eliminate the environmental pollution the world is facing today.

BIBLIOGRAPHY

1. Anonymous; Analytical Methods for Atomic Absorption Spectrophotometry, Perkin-Elmer.

2. Anonymous; Climatological Data, Environmental Data Service, National Oceanic and Atmospheric Administration, U.S. Department of Commerce.

3. Anonymous; Instructions for Mercury Analysis System, Perkin-Elmer Corporation, Norwalk, NJ.

4. Berman, E. (1972) Determination of Cadmium, Thallium, and Mercury in Biological Materials by Atomic Absorption, Atomic Absorption Newsletter, 6 57-60.

5. Biesinger, K.E. and G.M. Christensen, (1972) Effects of Various Metals on Survival, Growth, Reproduction, and Metabolism of Daphscia magna, J. Fish. Res. Bd. Canada 29 1691.

6. Brooks, R.R. and M.G. Rumsby (1965) The Biogeochemistry of Trace Element Uptake by Some New Zealand Bivalves, Limnol. Oceaogr. 10 521.

7. Brown, B. and M. Ahsanullah, (1971) Effect of Heavy Metals on Mortality and Growth, Mar. Poll. Bull. 2 182.

8. Bryan, G.W., (1973) The Occurrance and Seasonal Variation of Trace Metals in the Scallops Pecten mascimus (L.) and Chamys operculanis (L.), J. Mar. Biol. Ass. 53 145.

9. Burch, P.G., (1972) Development of a Flameless Atomic Absorption Assay for Mercury in Biological Materials and Levels of Mercury Detected in Striped Bass (Roccus Saxatilis) collected in the San Joaquin Delta (M.S. Thesis) University of the Pacific.

10. Calabrese, A., R.S. Collier, D.A. Nelson, and J.R. MacInnes, (1973) The Toxicity of Heavy Metals to Embryos of American Oyster Crassastrea virginica, Marine Biology 18 162.

11. Clarkson, T., (1972) The Pharmacology of Mercury Compounds, Ann. Rev. Pharmacol. 12 375.

12. Connell, J.H., (1970) A Predator-Prey System in the Marine Intertidal Region 1 Balanus glandula and Several Species of Thais, Ecological Monographs 40 49.

13. Cunningham, P. and M. Tripp, (1973) Accumulation and Depuration of Mercury in the American Oyster Crassostrea virginica, Mar. Biol. 20 14.

14. DeSoete, D., R. Gijbels, and J. Hoste, (1972) Neutron Activation Analysis, Wiley-Interscience, New York 34 1.

15. Eisler, R., (1971) Cadmium Poisoning in Fundulus heteroclitus (Pisces cyprinodontidae) and Other Marine Organisms, J. Fish. Res. Bd. Canada 28 1225.

16. Eisler, R., G.E. Zaroogian, and R.J. Hennekey, (1972) Cadmium Uptake by Marine Organisms, J. Fish. Res. Bd. Canada 29 1367.

17. Feder, H.M., (1959) The Food of the Starfish Pisaster ochraceus along the California Coast, Ecology 40 721.

18. Giese, A.C., (1966) Lipids in the Economy of Marine Invertebrates, Physiol. Rev. 46 244.

19. Gillespie, D.C., (1972) Mobilization of Mercury from Sediments into Guppies (Poccilia reticulata), J. Fish Res. Bd. Canada 29 1035.

20. Gillespie, D.C., and D.P. Scott, (1971) Mobilization of Mercuric Sulfide from Sediment into Fish Under Acrobic Conditions, J. Fish Res. Bd. Canada 28 1807.

21. Gladfelter, W.,(1973) Personal Communication, Pacific Marine Station, Dillon Beach, California.

22. Goodman, L.S., and A. Gilman, (1970) The Pharmacological Basis of Therapeutics, 4th ed. The Macmillan Company, New York 974.

23. Graham, D.L., (1972) Trace Metal Levels in Intertidal Mollusks of California, The Veliger 14 365.

24. Harger, J.R.E., (1971) Comparisons Among Growth Characteristics of Two Species of Sea Mussel, Mytilus edulis and Mytilus californianus, The Velinger 13 44.

25. Harger, J.R.E., (1972) Competitive Coexistance Among Intertidal Invertebrates, Am. Sci. 60 600.

26. Hatch, W.R., and Ott, W.L., (1968) Determination of Sub-Microgram Quantities of Mercury by Atomic Absorption Spectroscopy, Analyt. Chem. 40 2085.

27. Johnelo, A.G., and T. Westermark, (1969) Mercury Contamination of the Environment in Sweden, Chemical Fallout 221.

28. Kahn, H.L., (1967) Principles and Practice of Atomic Absorption, Adv. Chem. 73 183.

29. Kamps, L.R., R. Carr, and H. Miller, (1972) Total Mercury-Monomethylmercury Content of Several Species of Fish, Bull. Environ. Con. Tox. 8 273.

30. Kehoe, R.A., (1964) Metabolism of Lead Under Abnormal Conditions, Archives of Environmental Health 8 235.

31. Kimura, Y., and V.L. Miller, (1962) Mercury Determination at the Microgram Level by a Reduction Aeration Method of Concentration, Anal. Chim. Acta 27 325.

32. Klein, D.H., and E.D. Goldberg, (1970) Mercury in the Marine Environment, Environ. Sci. Technol. 4 765.

33. Lee, D.H.K., (1972) Metallic Contaminants and Human Health, Academic Press, New York.

34. Lovett, R.J., W.H. Gutenmann, I.S. Pakkala, W.D. Youngs, D.J. Lisk, G.E. Burdick, and E.J. Harris, (1972) A Survey of the Total Cadmium Content of 406 Fish from 49 New York State Fresh Waters, J. Fish Res. Bd. Canada 29 1283.

35. Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall, (1951) Protein Measurement with the Folin Phenol Reagent, J. Biol. Chem. 193 265.

36. Menge, B.A., (1972) Forging Strategy of a Starfish in Relation to Actual Prey Availability and Environmental Predictability, Ecol. Monogr. 42 25.

37. Paine, R.T., (1966) Food Web Complexity and Species Diversity, The Amer. Naturalist 100 65.

38. Pickering, Q.H., and M.H. Gast, (1972) Acute and Chronic Toxicity of Cadmium to the Fathead Minnow (Pimephales promelas), J. Fish Res. Bd. Canada 29 1099.

39. Pringle, B.H., D.E. Hissong, E.L. Katz, and S.T. Mulawka, (1968) Trace Metal Accumulation by Estuarine Mollusks, J. San Eng. Div. 94 455.

40. Ricketts, E.F., and J. Calvin, (1968) Between Pacific Tides, 4th ed. Stanford University Press, California.

41. Rivers, J.B., and J.E. Pearson, and C.D. Shultz, (1972) Total and Organic Mercury in Marine Fish, Bull. Environ. Con. Tox. 8 257.

42. Schwimer, S.R., (1973) Trace Metal Levels in Three Subtidal Invertebrates, The Veliger 16 95.

43. Scott, D.P., and F.A.J. Armstrong, (1972) Mercury Concentration in Relation to Size in Several Species of Freshwater Fishes from Manitoba and Northwestern Ontario, J. Fish. Res. Bd. Canada 29 1685.

44. Sjostrand, B., (1964) Simultaneous Determination of Mercury and Arsenic in Biological and Organic Materials by Activation Analysis, Analyt. Chem. 36 814.

45. Sprague, S., and W. Slavin, (1964) Determination of Very Small Amounts of Copper and Lead in KCl by Organic Extraction and Atomic Absorption Spectroscopy, Atomic Absorption Newsletter 3 37.

46. Storer, T.I., R.L. Usinger, R.C. Stebbins, J.W. Nybakken, (1972) General Zoology, 5th ed., McGraw-Hill, New York 483.

47. Swanson, R.G., (1973) Some Aspects of Mercury Accumulation by the Purple Shore Crab Hemigrapsus Nudus, (M.S. Thesis) University of the Pacific.

48. Thorpe, V.A., (1971) Determination of Mercury in Food Products and Biological Fluids by Aeration and Flameless Atomic Absorption Spectrophotometry, J. Assoc. Ott. Anal. Chem. 54 206.

49. Vallee, B.L., and D.D. Ulmer, (1972) Biochemical Effects of Mercury, Cadmium, and Lead, Ann. Rev. Biochem. 41 91.

50. Vernberg, W., and F. Vernberg, (1972) The Synergistii Effects of Temperature, Salinity, and Mercury on Survival and Metabolism of the Adult Fiddler Crab Uca pugilator, Fish. Bull. 70 415.

51. Vernberg, W., and J. O'Hara, (1973) Temperature-Salinity Stress and Mercury Uptake in the Fiddler Crab Uca pugilator, J. Mar. Biol. Assoc., 29 1491.

52. Windom, H., R. Stickney, R. Smith, D. White, and F. Taylor, (1973) Arsenic, Cadmium, Copper, Mercury, and Zinc in Some Species of North Atlantic Finfish, J. Fish. Res. Bd. Canada 30 275.

53. Wisely, B., and R.A.P. Blick, (1967) Mortality of Marine Invertebrate Larvae in Mercury, Copper and Zinc Solutions, Aust. J. Mar. Freshwater Res. 18 63.

54. Zitko, V., B.J. Finlayson, B.J. Wildish, J.M. Anderson, and A.C. Kohler, (1971) Methylmercury in Freshwater and Marine Fish in New Brunswick, in the Bay of Fundy, and on the Nova Scotia Banks, J. Fish. Res. Bd. Canada 28 1285.